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نموذج رقم (١٦)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية وقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الدكتوراه

أنا الطالب: ابراهيم رجب دبور   الرقم الجامعي: (٩٠٥٠١٠٥)
تخصص: التغذية والتصنيع الغذائي   الكليـــة: الزراعة

عنوان الاطروحة: الصفات الكيماوية لزيت بذور الرمان وتأثيره على الصورة لبيدات الدم والسعة المضادة للأكسدة في جرذان بالغة مغذاة بأطعمة معززة بالكوليسترول.

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد اطروحات الدكتوراه عندما قمت شخصيا" باعداد اطروحتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الأطروحات العلمية. كما أنني أعلن بأن اطروحتي هذه غير منقولة أو مستلة من أطاريح أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

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CHEMICAL PROPERTIES OF MILK THISTLE SEED OIL AND ITS EFFECT ON SERUM LIPID PROFILE AND ANTIOXIDANT CAPACITY IN ADULT RATS FED CHOLESTEROL – SUPPLEMENTED DIETS

By

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This Dissertation was Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Nutrition and Food Science

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COMMITTEE DECISION

This Dissertation (Chemical Properties of Milk Thistle Seed Oil and Its Effect on Serum Lipid Profile and Antioxidant Capacity in Adult Rats Fed Cholesterol – Supplemented Diets) was Successfully Defended and Approved on August 8, 2011.

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DEDICATION

To almighty Allah ……. who guided me throughout my life time

To my parents ……. for their unflagging love and support

To my great wife ……. Wafa

To my lovely kids………….Khalid, Rand and Raghad

the source of joy and happiness in my life

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

%	Percentage
ABAP	2,2- Azobis (2- Aminopropane)
ABTS	2,2-Azinobis- (3-ethylbenzothiazoline-6- sulfonic acid)
AI	Atherogenic Index
ALP	Atherogenic Lipoprotein Phenotype
AOAC	Association Of Official Analytical Chemists
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CETP	Cholesteryl Ester Transfer Protein
CHD	Cornary Heart Disease
CM	Chylomicron
CUPRAC	Cupric Reducing Antioxidant Capacity
CV	Coefficient Of Variation
CVD	Cardiovascular Disease
DAD	Photodiode- Array Detection
DPPH	2,2-Di(4-tert-octylphenyl)-1- picrylhydrazyl
ET	Electron Transfer
FAMES	Fatty Acid Methyl Esters
FER	Food Efficiency Ratio
FID	Flame Ionization Detector
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
GLC	Gas Liquid Chromatography
HAT	Hydrogen Atom Transfer
HDL-C	High-Density Lipoprotein- Cholesterol
HMG-COA	3- Hydroxyl-3-Methylglutaryl Coenzyme A
HPLC	High-Performance Liquid Chromatography
IC_{50}	Concentration to Inhibit The Free Radical $DPPH^{\bullet}$ in a 50%
IDL	Intermediate Density Lipopntein
IHD	Ischemic Heart Disease

JFDA	Jordan Food And Drug Administration
Kg	Kilogram
L	Liter
LCAT	Lecithin- Cholesterol Acyltransferase
LDL-C	Low-Density Lipoprotein – Cholesterol
LPL	Lipoprotein Lipase
MDA	Malondialdehyde
MeOH	Methanol
mEq	Milli Equivalent
mG	Milligram
mL	Milliliter
mmol	Millimole
MT	Milk Thistle
MTSO	Milk Thistle Seed Oil
MUFA'S	Monounsaturated Fatty Acids
NFE	Nitrogen Free Extract
nm	Nanometer
ORAC	Oxygen Radical Absorbance Capacity
OSI	Oxidative Stability Index
PUFA'S	Polyunsaturated Fatty Acids
PV	Peroxide Value
ROS	Reactive Oxygen Species
SD	Standard Deviation
SEM	Standard Error Of The Mean
SFA'S	Saturated Fatty Acids
SPL	Split Injector Port
TAC	Total Antioxidant Capacity
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid- Reactive Substances
TBHQ	Tert-Butyl Hydroquinone
TC	Total Cholesterol
TEAC	Trolox ® Equivalent Antioxidant Capacity

TG	Triglyceride
TMS	Trimethylsilyl
TPC	Total Phenolic Contents.
TRAP	Total Peroxyl Radical- Trapping Antioxidant Parameter
USFA'S	Unsaturated Fatty Acids
UV	Ultra Violete
VLDL	Very Low Density Lipoprotein
X-(Fe^{IV}=O)	Ferrylmyoglobin
µL	Micro Liter

CHEMICAL PROPERTIES OF MILK THISTLE SEED OIL AND ITS EFFECT ON SERUM LIPID PROFILE AND ANTIOXIDANTS CAPACITY IN ADULT RATS FED CHOLESTEROL – SUPPLEMENTED DIETS

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Abstract

This study aimed to examine the chemical characteristics of cold-pressed milk thistle seed oil (MTSO) and its effect on *in vivo* serum lipid profile, lipid peroxidation product (MDA) and total antioxidant capacity (TAC).

Acidity and peroxide value (PV) were determined by chemical standard titration method and oxidative stability index (OSI) was determined by Rancimate. Using (GLC), fatty acid composition, phytosterols and squalene were measured. While, α-tocopherol content was determined by (HPLC). The total phenolic content, radical-scavenging capacity and TAC were determined spectrophotometrically. The *in vivo* effect of MTSO on serum lipid profile, MDA and TAC was carried out to investigate the long-term effect of feeding two levels of MTSO in experimental diets with and without cholesterol. Sixty-four adult male Sprague-Dawley rats were divided into eight groups. Four groups were on free cholesterol diets with either 4 % MTSO, 8% MTSO, 4% control (soybean oil) or 8% control oil. The other 4 groups were fed 1% cholesterol with percentages of oils similar to the first four groups for 12 weeks. Fasting serum levels of total cholesterol (TC), high density lipoprotein–cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and TAC were determined by enzymatic –colorimetric method using standard kit procedure. Serum MDA was determined by thiobarbituric acid reactive substances (TBARS) assay method using spectrophotometery. Body weights, liver weights and food intakes were also recorded.

The chemical characteristics of cold-pressed MTSO were as follows: acidity (0.64%), PV (0.34 meq O_2 / kg of oil), OSI (55.7 hr and 12.9 hr at 80 Ċ and 100 Ċ, respectively), fatty acid composition (19.5%, 22.9% and 57.6% for SAT, MUFA and PUFA respectively), phytosterol (2520 mg/kg), squalene (9.35 mg/kg), α-tocopherol content (237.4 mg/kg), total phenolic content (1.16 mg GAE/g oil), radical-scavenging capacity against stable DPPH radical (IC50=3.34 mg/ml) and TAC (2.29 mmol/L).

Feeding two levels of MTSO and control oil diet to various cholesterol –free and cholesterol –supplementd rat groups resulted in insignificant (P>0.05) changes in weight gain, accumulative food intake and food efficiency ratio. However, liver weights were

significantly different (P<0.05) in 8 % MTSO and 8% control of cholesterol-supplemented diet compared to other diet groups. Irrespective of cholesterol, body weight gain, liver weights, accumulative food intake and food efficiency ratio were insignificantly different (P>0.05). When taking cholesterol into account, liver weights were significantly different (P<0.05) in 8% of MTSO and control diet in cholesterol-supplemented diets. Irrespective of oil type and irrespective of oil %, similar results were obtained. Regarding serum lipoproteins there were significant differences (P<0.05) in TC in 8% MTSO and 8% control cholesterol-supplemented diets when compared with 8%control cholesterol-free diets. Also LDL-C of cholesterol-supplemented diet groups were significantly higher (P<0.05) than cholesterol-free diet groups. An interesting result was the significantly high levels (P<0.05) of HDL-C in rats fed cholesterol-free diet with MTSO 4% and 8% when compared to cholesterol –supplemented diets. There was a significantly higher (P<0.05) HDL-C/LDL-C ratio for cholesterol-free diets than cholesterol-supplemented diets. Subsequently, high significant difference (P<0.05) was observed in AI values at 8% cholesterol-supplemented diet when compared with cholesterol-free diet. At 4% oil (irrespective of oil type) in presence of cholesterol, values of serum TC, LDL-C, TC/TG and AI were significantly lower (P<0.05) than values at 8%. With respect to blood TG level, no significant variations (P>0.05) were found in MTSO diets when compared with control diets whether with or without cholesterol except for the 4% cholesterol- free control diet group which tended to have higher TG value (P<0.05) when compared with all diet groups. Moreover, irrespective of oil type, TG concentration tended to be obviously higher in cholesterol-free diets at 4% oil. MDA showed clearly high levels at 8% cholesterol-free control diet when compared with other cholesterol-free diets. Additionally the 8% cholesterol-supplemented control diet was higher than that of 8% cholesterol-supplemented MTSO diet (P<0.05). Irrespective of cholesterol, similar results were obtained regarding MDA. While in presence of cholesterol at 4% and 8% fat, MDA levels were higher (P<0.05) when compared with 4% without cholesterol. When exploring the effect of oil type with cholesterol (irrespective of fat %) MDA was also high in control oil with or without cholesterol. This shows that higher level of MDA was in 8% control oil with or without cholesterol. Regarding the TAC, it was higher (P<0.05) in 4% cholesterol-free MTSO diet when compared to 8% cholesterol-free control diet and 4% cholesterol –supplemented MTSO diet. Irrespective of oil % higher TAC was found in cholesterol-free MTSO diet.

In conclusion, these data suggest that MTSO may serve as dietary source of high PUFA and MUFA, phytosterols, phenolic compounds and natural antioxidants and can be an attractive candidate for use in healthy food preparations mixed with other vegetable oils or alone. Based on the results obtained, the % of oil and presence of cholesterol were the main factors affecting the lipid profile, calculated AI, lipid peroxidation and TAC. Consequently, the MTSO and soyabean oil (control) had the same effect on these variables. With respect to *in vivo* lipid peroxidation, MTSO was clearly superior to soybean oil (control).

Introduction

Oil seeds have been part of the human diet for a long time and their production has shown a remarkable increase in the last few decades (Tuberoso *et al*., 2007; Parry *et al*., 2008). Edible seed oils are a group of important food ingredients. Seeds oil, rich in factors beneficial to health, are of high demand because of consumers interest in disease prevention and health promotion through improved diets (Gorinstein *et al*., 2003; Bail *et al*., 2008). These beneficial factors may include high content of monounsaturated fatty acids (MUFA'S), polyunsaturated fatty acids (PUFA), tocopherols, carotenoids and antioxidant phenolic compounds (Gorinstein *et al*., 2003; Garjani *et al*., 2009).

In the recent years, there has been a growing interest in the oils of non-traditional seeds such as the oils of flaxseeds, safflower seeds, pumpkin seeds, grape seeds, pomegranate seeds and milk thistle seeds. The potential use of these oils is gaining importance as nutraceuticals and phytoceuticals for improving human nutrition and health and for disease prevention (Abolfazl Fadavi *et al*., 2006; Bail *et al*., 2008; Hauenschild *et al*., 2010; Shaker *et al*., 2010).

Milk thistle (MT) *Silybum marianum*, L. Gaertn is an annual wild plant of the *Compositeae* family which is native to the Mediterranean area, including Jordan, and North Africa (Tukan *et al*., 1998; Fathi-Achachlouci and Azadmard-Damirchi, 2009). It grows wild throughout Europe, some parts of the United States and Australia. MT can be also cultivated (Barreto *et al*., 2003).

In recent decades, it has been suggested that certain substances in MT seeds, especially a flavonoid called silymarin, protects the liver from toxic chemicals and drugs such as acetaminophen which can cause liver damage in high doses. Silymarin was found to have an

antioxidant and anti-inflammatory properties that may help liver to repair itself by growing new cells (Jacobs *et al*., 2002; Nyiredy *et al*., 2008; Shaker *et al*., 2010).

Cardiovascular disease (CVD) is still responsible for the most common cause of death for both males and females worldwide (Houenschil *et al*., 2010). Mortality statistical data from Jordan Ministry of Health indicates that 38.2% of deaths in 2003 were attributed to CVD (Khader *et al*., 2010). Dyslipidemia, which is closely linked to the pathophysiology of CVD, is a key independent modifiable risk factor for CVD (Hammoudeh *et al*., 2008). Brown *et al* (2009) and Khader *et al.* (2010) found that hypercholesterolemia and hyperglyceridemia are highly prevalent in Jordan with a pattern consistent with that of United States and other developed countries.

Many studies showed that different dietary lipids can modulate plasma cholesterol level, depending on their fatty acid composition. High levels of dietary saturated fatty acids (SFA) or *trans* fatty acids are associated with the formation of atheroscleorotic plaque. However, intakes of MUFA or oleic acid and PUFA of the n-3 series and n-6 series are associated with decreased risk of CVD (Moon *et al*., 2001; Cintra *et al*., 2006). Several clinical trials indicated that consumption of 13% to 21% dietary energy from PUFA decreased total plasma cholesterol by 13% to 15% and decreased CVD events by 25% to 43% (ADA, 2007).

It has been found in a number of studies that MT seeds contain a relatively high content of oil (20-26%) (Abu-Jadayil *et al*., 1999, Abu-Rajouh and Takruri. 1999; Parry *et al*., 2006; Fathi-Achachlouei and Azad mard –Damirchi, 2009). The main fatty acids in MT seed oil were found to be linoleic acid, oleic acid, palmitic acid and stearic acid. The oil has also been suggested to be edible.

There are few data indicating capability of MT seed or its oil to modulate and positively affect lipoprotein metabolism and reduce the risk of CVD (Skottova *et al*., 2003; Sobolova *et al*., 2006). The health importance of milk thistle seed oil was suggested to be due to the antioxidant activity of phytochemcials compounds against CVD and lipid peroxidation. These compounds include polyphenols, phytosterols, chlorophylls, squalene, tocopherols and carotenoids which represent a minor component in plant oils (Baba *et al*., 2000; Gorinstein *et al*., 2003; Visavadiya and Narasimhacharge, 2008).The USFA and phytochemical compounds also affect the pro-and /or antioxidative processes in the oils (Parry *et al*., 2006; Tuberoso *et al*., 2007; Bail *et al*., 2008; Fathi-Achahlouei and Azadmard-Damirchi, 2009).

The aims of the previous studies were to demonstrate a pharmaceutical effect of MT seed as based on the use of its extract, derivates, or whole seed powder. To the best of our knowledge, no studies were conducted to investigate the chemical characteristics of the oil of MT seeds grown in Jordan and its effect on blood lipid profile. Information from controlled intervention trials in humans or animals regarding the *in vivo* effects of MT seed oil on the antioxidant capacity is not available.

It is hypothesized that regular incorporation of cold-pressed MT seed oil in the diet will favorably affect blood lipid profile and antioxidant status in rats, and the level of the oil and/or the cholesterol content of the diet may influence the extent of this effect. Thus, the aim of this study will be to:

1) Examine the chemical characteristics of the oil extracted from MT seeds, including fatty acid composition, peroxide value, total acidity, radical scavenging capacity, oxidative stability index, total antioxidant capacity and content of total phenols, tocopherols, squalene and phytosterols.

2) Study the effect of MT seed oil (MTSO) on *in vivo* serum lipid profile, total antioxidant capacity and lipid peroxidation product, namely malondialdehyde in adult rats fed cholesterol – supplemented diets.

Literature Review

1. Blood Lipids and Lipoproteins

1-1 Introduction

Lipids, being insoluble in the aqueous environment of the blood, need to be combined with proteins for their transport in the circulation. Aggregates of hydrophobic molecules, particularly triglycerides and cholesteryl esters, are stabilized by non-covalent forces with a hydrophilic coat of phospholipids and proteins, and the amphipathic unesterfied cholesterol. The resulting particles are called lipoproteins (Gurr *et al*., 2002). The protein moieties, known as apoproteins have more than stabilizing role; they confer specificity of the particles allowing them to be recognized by specific receptors on cell surfaces, they also regulate the activity of some enzymes involved in lipoprotein metabolism (Gurr *et al*., 2002; Schultz and Liebman, 2002). There are several types of lipoproteins with different chemical compositions, physical properties and metabolic functions, but their common role is to transport lipids from one tissue to another, so as to supply the lipid needs of the different cells (Whitney and Rofles, 2008)

1-2 Classification

Different lipoproteins may be classified in a number of ways according to their origins, major functions, composition, physical properties or the method of these isolation, they differ in the proportions and types of lipids (triglycerides, esterfied and non –esterified cholesterol and phospholipids). These compositional differences influence the density of the particles, in general; the higher the lipid to protein ratio, the larger the particle and the lower its density (Gurr *et al*., 2002). Lipoproteins are separated and analyzed either as based on their density where ultracentrifugation is used: or based on their apoprotein composition where electrophoresis is used (Schultz and Leibman, 2002). Four classes of plasma lipoproteins exist

in normal fasting humans; very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoprotein (LDL) and high density lipoproteins (HDL). In the postabsorptive period a fifth type called chylomicrin (CM) is also present (Mudd *et al.*, 2007; Whitney and Rolfes, 2008). Composition and characteristics of the human lipoproteins are summarized in table 1.

1.3 Metabolism and regulation

It is convenient to describe lipoprotein metabolism as consisting of three interrelated pathways. In the exogenous pathway; after hydrolysis and re-esterification of dietary fat in the gut, the re-esterfied triglycerides are secreted from the enterocytes as triglycerides-rich CM particles into the lymphatic system, and then to the blood stream. Lipoprotein lipase (LPL) in the endothelial lining of capillaries of the adipose tissue, skeletal and cardiac muscle and mammary gland, hydrolyses about 80% of the triglyceride particles (Gurr *et al*., 2002). The generated fatty acids diffuse into the adjacent tissues, while the remnant CM particles are taken up by the cell-surface receptors on hepatocytes. Decomposition of the remnant CM to its components, results in the release of amino acids and cholesterol in hepatocytes. Cholesterol could be then excreted into bile, or converted into bile acids, or incorporated into VLDL or esterified with long-chain fatty acids and stored in the liver (Whitney and Rofles, 2008). The exogenous pathway is regulated mainly by the rate of entry of dietary fat, but the tissue disposition of the dietary fatty acids is regulated by tissue-specific regulation of LPL activity (Gurr *et al*., 2002).The secretion of VLDL from the liver, and its further metabolism, is described as the endogenous pathway. The fatty acids produced from CM hydrolysis are taken up by the liver and the adipose tissues to produce the endogenous triglyceride-rich. LipoproteinVLDL, which is the main lipoprotein secreted from liver (Harvey and Champe.2005).

Table (1): Composition and characteristics of the human lipoproteins.

Classes / Characteristics	Chylomicrons	VLDL	IDL	LDL	HDL
Protein (% particle mass)	2	7	10	20	50
Triglycerides (TG) (% particle mass)	83	50	40	10	8
Cholesterol (% particle mass; free + esterfied)	8	22	30	48	20
Phospholipids (% particle mass)	7	20	20	22	22
Particle mass (Dalton	$0.4\text{-}30 \times 10^6$	$10\text{-}100 \times 10^6$	4.5×10^6	$2\text{-}3.5 \times 10^6$	$1.74\text{-}3.6 \times 10^5$
Density range (g mL^{-1})	<0.95	0.95-1.006	1.006.1.019	1.019-1.063	1.063-1.210
Diameter (nm)	80-1000	30-90	25-40	18-22	5-12
Apolipoproteins	AI, AII, AIV, B-48, CI, CII, CIII, E	B100, CI, CII, CIII, E	B100, E	B100	AI, AII, AIV, CI, CII, CIII, D, E
Place of synthesis	Intestine	Liver and intestine	Liver	Liver and intestine	liver
Function	Transport of dietary TG to plasma, tissues and cells	Transport of endogenous TG to cells	-Continue transport of endogenous TG –LDL precursor	Transport cholesterol to peripheral tissue	Transport free cholesterol to liver for catabolism
Correlation to atherosclerosis	Positive	Positive	Positive	Highly Positive	Negative

Adapted from (Gurr *et al*., 2002; Schultz and Liebmna, 2002).

Then, similar to the exogenous pathway, VLDL particles successively interact with LPL in capillaries, as a result their core triglycerides are partially hydrolyzed to form LDL. IDL, which may be considered as the VLDL remnant, is then taken up by the liver where it is partially degraded by hepatic lipase to form LDL (Schultz and Liebmna, 2002).

Each type of lipoproteins has its distinguishable apolipoproteins as described in Table 1. The major site for removal of LDL particles from circulation is normally the liver, though the LDL-receptor is a mechanism for delivering cholesterol to most cells. The expression of LDL-receptors is closely regulated to maintain cellular cholesterol homeostasis. Thus, the endogenous pathway is a means for delivering fatty acids to tissues via LPL, and cholesterol via the LDL-receptor (Gurr *et al.*, 2002).

The third pathway is that of HDL metabolism, which may be regarded as a reverse of the LDL pathway of cholesterol delivery to tissues. Immature lipid-poor HDL particles, synthesized in liver and secreted to blood, interact with cell membranes to gain unesterfied cholesterol. This cholesterol becomes esterfied with a long-chain fatty acid through the action of the enzyme lecithin-cholesterol acyltransferase (LCAT), which is associated with HDL particles, in the presence of apoprotein AI as a co-factor (Schultz and Liebmna, 2002). The formed cholesteryl ester moves to the core of the HDL_3 particle, which swells and becomes spherical as more cholesterol is acquired and esterified to form mature, lipid rich HDL_2 particle. This is probably the origin of the bulk of HDL-C in plasma, although some is acquired from the surface components of CM and VLDL particles when their triglycerides are hydrolyzed by LPL (Gurr *et al.*, 2002). To complete the pathway of reverse cholesterol transport, HDL-C must return to the liver either directly via a hepatic receptor, or by being exchanged for triglycerides from CM and VLDL via the action of cholesteryl ester transfer protein (CETP). These triglyceride-rich lipoprotein particles then

carry this cholesterol to the liver for receptor-mediated uptake (Gurr *et al*., 2002; Mudd *et al*., 2007).

1.4 Lipoproteins and cardiovascular diseases

1.4.1 Pathophysiology

Cardiovascular disease (CVD) is a broad term for a number of conditions involving pathological changes in blood vessels associated with heart and brain. The major causes of death among CVD are ischaemic heart disease (IHD), or coronary heart disease (CHD), and ischaemic stroke. The common chronic condition underlying these two diseases is atherosclerosis (Mudd *et al*., 2007; Micallef and Garg, 2009).

Atherosclerosis is an irregular thickening of the walls of arteries that supply blood to the heart or brain or peripheral tissues. So, these arteries become narrower and prevent the normal circulation. The situation is exacerbated if platelets aggregated and blood clot (thrombus) was formed at the site of narrowing, as these may lead to the blockage of an artery, causing a heart attack (myocardial infraction) or a brain stroke. Peripheral vascular diseases involve blood vessels in limbs, and although that is not a major cause of mortality, it is associated with considerable morbidity and distress for patients (Gurr *et al*, 2002). The atherosclerotic plaques consist of proliferation of smooth muscle cells, cell debris, connective tissues and mucopolysaccharides fat-filled foam cells, in which the predominant lipids are cholysteryl ester and cholesterol monohydrate, and deposits of calcium, fibrin and other plasma proteins beneath (Micallef and Garg, 2009)

It is well-documented that the risk factors for CHD are usually divided into two categories. Non-controllable factors including age, sex and family or genetic history and controllable factors including cigarette smoking, high blood pressure, elevated serum

cholesterol levels, body weight, stress, dietary intake and exercise (Whitney and Rofles. 2008). It is well-established that certain plasma lipids and lipoproteins play a significant role as risk factors for the development of atherosclerosis. The athergoneic lipoprotein phenotype (ALP) describes a common collection of abnormalities in plasma lipoproteins which confer increased risk of CHD upon normal, healthy individuals (Mudd *et al*., 2007). The ALP is characterized by a moderately raised level of serum TG, a low level of HDL and a predominance of abnormally small, dense LDL particles. Levels of TC and LDL-C are typically normal or only moderately raised, while the concentrations of TG-rich lipoproteins (CM or VLDL) are usually elevated (Gurr *et al*., 2002; Mudd *et al*., 2007).

Several theories were proposed to explain the increased atherogenicity of small dense LDL. It was suggested that this LDL subclass has prolonged residence time in the circulation, owing to its slow removal by cellular LDL receptors. These receptors' pathway accounts for about 50% of overall LDL catabolism, thus increasing the time available for its infiltration and trapping into the arterial wall (Mudd *et al*., 2007). Also small dense LDL particles show an increased susceptibility to lipid peroxidation, which leads in turn to peroxidation of the apoprotein B 100. This alters the affinity of the LDL particles from LDL-receptors to scavenger receptors of the macrophages. Lipid uptake by the scavenger receptor pathway is not subjected to a feedback regulation by cellular cholesterol content. Therefore, the macrophages may engulf large amount's of lipid, giving rise to the formation of foam cells, which is a characteristic of the atherosclerotic plaque (Gurr *et al*., 2002: Niki, 2010).

High-density lipoprotein functions as the initiator of reverse cholesterol transport, promoting the clearance of cholesterol from peripheral tissues to the liver, either directly by binding to cell surface receptors via apoprotein AI, or indirectly via transport of

cholesterol to other lipoproteins. This role underlies the inverse relationship between the plasma level of HDL-C, particularly the less dense HDL, and CHD. Low plasma HDL-C is usually associated with an increased TG concentration. In fact, the metabolism of HDL is closely linked to that of the TG-rich lipoproteins, they exchange components and HDL is mostly derived from the lipolysis of CM and VLDL (Bartosz, 2003).

Recent studies indicate that CHD may be associated with a reduced efficiency in the metabolism of TG-rich lipoproteins. Hypertriglycerdemia is a relative inability to clear CM and VLDL from blood circulation, an increase in cholesterol ester transfer from HDL to IDL occurs subsequently leading to enhanced formation of small dense LDL particles and lowered HDL levels, which are characteristics of the ALP (Micallef and Garg, 2009).

Several attempts have been made to favorably affect blood lipid profile. The ability to reduce plasma cholesterol by pharmacological means was dramatically improved with the introduction of the 'statin' drugs, inhibitor of a 3-hydroxyl-3- methylglutaryl coenzyme A (HMG-CoA) reductase particularly in the liver, thereby inhibiting cholesterol synthesis (Gurr et al., 2002:Micallef and Garg, 2009,). However, attention must be drawn to dietary modification as an effective mean to achieve a cardioprotective lipoprotein profile.

1.4.2 Relation to diet

The atherogenic lipoprotein phenotype (ALP) is likely to be developed through a complex interaction between dietary factors and specific lipoprotein genes, the expression of which is susceptible to certain dietary stimuli (Gurr et al., 2002). Epidemiological studies have showed that level of dietary fat intake is positively correlated with the average serum cholesterol. Also, as the amount of dietary cholesterol entering the body is increased, there is an expansion, of the cholesterol pool in the liver and down regulation of

LDL-receptors that are primarily responsible for clearing LDL-C from the circulation (Bartosz, 2003). A number of different investigators demonstrated that in addition to total fat, fatty acid composition of diets is a determinant of plasma lipid levels. Generally, the replacement of dietary saturated fatty acids with unsaturated ones is accompanied by a lowering of fasting serum TG, TC and LDL-C even when dietary cholesterol intake is kept constant; (Kattan and Mensink,1994, Wilson *et al*,2000, Bergmann *et al*,2005, Kritchevsky and Chen,2005). With regards to individual saturated fatty acids (SFA), they may not be equally cholesterolemic. In a strictly controlled human feeding study for Tholstrup *et al*. (1994), stearic acid (18:0) consumption, which substantially correlates with SFA intake, resulted in a significant reduction in plasma TC, LDL-C and HDL-C levels, compared to other SFA in foods, namely lauric acid (12:0) myristic acid (14:0), and palmitic acid (16:0). However, the effect of any fatty acid is highly dependent on both, the metabolic status of the individual and the other dietary fatty acids present (Billett *et al*., 2000; Kritchevsky *et al*., 2001)

Monounsaturated fatty acids (MUFA), like oleic acid (18:1), are known to have a hypocholesterolemic effect because they enhance apoprotein B clearance. They are also reported to decrease the fasting plasma triglycerides (Baba *et al*., 2000; Grundy, 2006). On the other hand, polyunsaturated fatty acids (PUFA) (both n-6 and n-3 series) suppress the production of VLDL, leading to a hypocholesterolemic effect (Simopoulos, 2002; Makni *et al*., 2008). In many studies, the replacement of saturates with linoleic acid (18:2, n-6 PUFA) reduced both LDL-C and HDL-C levels (Makni *et al*., 2008; Bhupathiraju and Tucker, 2011). The double bond in the naturally occurring dietary unsaturated fatty acids is usually in the *cis* configuration, but when it occurs in the *trans* configuration, a large rise in blood LDL-C is induced with a fall in HDL-C levels (Mudd *et al*., 2007).

The effect of dietary fats on serum cholesterol is widely assumed to be due solely to the fatty acids and the cholesterol they contain. Phytosterols, sterol oxidation products and sterol precursors, are however, present in dietary fats. These trace components act within the intestine to reduce cholesterol absorption and lower LDL-C concentration without being absorbed themselves (Micallef and Garg, 2009).

1.4.3 Dietary recommendations

The nutrition committee of the American Heart Association (AHA) recommends seven major dietary guidelines for the general population. These guidelines place a greater emphasis on the adoption of healthy eating patterns, behaviors and dietary fat intake (AHA, 2006). The recommendations include: (1) achieving and maintaining a healthy eating patterns that includes foods from each of the major food groups, (2) achieving and maintaining a healthy body weight, (3) achieving and maintaining a desirable blood cholesterol and lipoprotein profile, (4) achieving and maintaining a normal blood pressure, (5) achieving and maintaining a normal blood glucose level, (6) be physically active and (7) avoid use of and exposure to tobacco products (AHA, 2006).

With regard to the control of blood cholesterol and lipoproteins profile, the AHA recommends that the general population should reduce the total intake of cholesterol-raising fatty acids (saturated fat and *trans* fatty acids) to less than 10% of total energy intake, while people at higher risk of CVD shouldn't consume more than 7% of their energy intake from these fatty acids. This can be accomplished by limiting the intake of meat, high-fat dairy products and highly saturated fats and oils. In addition, partially hydrogenated vegetable oils, like vegetable shortening and hard margarine, should be substituted by soft or liquid margarine. Foods that are high in cholesterol should be reduced so that the amount of cholesterol is limited to less than 300 mg/day for the general

population, and to less than 200 mg/day for populations at higher risk of CVD (AHA, 2006).

Polyunsaturated fatty acids (especially n-3 series), MUFA and carbohydrates result in similar reduction of LDL-C levels when substituted for saturated fat; however, it is recommended not to derive more than 60% of calories from carbohydrates, because this may lead to elevation of TG and a decrease in HDL-C levels in susceptible individuals. The carbohydrate of choice is from unprocessed grains and high-fiber foods. Soluble fibers showed a reduction in LDL-C, especially in hypercholesterolemic individuals. Dietary fibers present in fruits, vegetables, and whole grains, are good sources of vitamins, minerals, and other potentially cardioprotective compounds, such as antioxidants (Dimitrios, 2006, Bhupathiraju and Tucker, 2011).

Dietary antioxidants include carotenoids, vitamin C, vitamin E, and polyphenols (Parry et al., 2005; 2006; 2008, Dimitrios, 2006, Whitney and Rolfes, 2008). The main polyphenol dietarary sources, which contribute to a total of about 1 g/day, are fruits and beverages (fruit juice, wine, tea, coffee, cocoa, and beer) and, to a less extent vegetables, dry legumes and cereals (Dimitrios, 2006; Whent *et al*., 2010). Phenolic acids account for about one third of the total intake and flavonoids account for the remaining two thirds (Whent *et al*., 2010). Many studies indicated that a high intake of fruits and vegetables is associated with a reduced CVD risk through the beneficial combination of antioxidants, fiber, potassium, magnesium and other phytochemicals (Dimitrios, 2006; Moore *et al*.,2005; Bhupathiraju and Tucker, 2011). Thus, optimal nutrition should contain polyphenols. However, because a valid procedure to identify polyphenols in plasma is not available, analysis of plasma antioxidants capacity is suggested as a suitable index to define the optimal nutritional intake (Niki, 2010).

2- Oxidative Stress: Prooxidants and Antioxidants

2-1 Introduction:

In human, an imbalance between prooxidant and antioxidant in favour of the prooxidants, potentially leading to tissue injury, is termed "oxidative stress". In this condition the rate of prooxidants production increase and / or the antioxidant mechanisms are impaired (Whitney and Rofles, 2008; Niki, 2010).

Prooxidants (such as free radicals) are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological condition and they are also generated by different types of radiation, air pollution and tobacco smoke (Dimitrios, 2006; Whitney and Rolfes, 2008).

Defense mechanisms against the effect of excessive prooxidation are provided by the action of various antioxidants. In their definition of the antioxidants terms, Antolovich *et al*. (2002) and Niki(2010) state that an antioxidant is "any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate." This definition includes compounds of a non-enzymatic as well as an enzymatic nature.

2-2 Prooxidants

2-2-1 Reactive Oxygen Species – Physiology

Free radicals are highly reactive substances formed in the body cells as a result of metabolic process including, oxidation – reduction reactions process (McCord, 2000; Niki, 2010). In 1954 Gerschman *et al*., first proposed the theory of free radical formation (Gerschman *et al.,* 2001). A free radical is a molecule with one or more unpaired electrons

in its outer orbital. Many of these molecular species are oxygen (and sometimes nitrogen) centered. Oxygen free radicals and its non-radical products are associated with reactive oxygen species (Antolovich *et al*. 2002; Niki, 2010).

Free radicals are highly reactive, unstable molecules that react rapidly with adjacent molecules via a variety of reactions including: hydrogen abstraction (capturing), electron donation and electron sharing (Mc Cord, 2000). For example, the process of capturing an electron involves reacting with a donor molecule, which loses an electron and is said to have been oxidized (Lee and Min, 2004). The oxidized donor molecule then has the capacity to oxidize other molecules and, thus, sets up a chain reaction (self-propagation reactions). Although free radicals play an essential role in the body, they also can react with DNA, protein or lipids in the cell membrane and cause damage (Del Rio *et al*., 2005).

2-2-1-1 Reactive Oxygen Species - Sources

Reactive Oxygen Species are found intracellularly and extracellularly and may be produced endogenously or arise from exogenous sources (taken from the environment). Important sources of endogenous free radicals include prooxidative enzyme systems (e.g. lipoxygenase), drugs and their metabolites, pollutants, and other chemicals and toxins. While some of these are directly toxic, many others generate free radical fluxes via the very metabolic processes that the body uses to detoxify them (Mc Cord, 2000; Niki, 2010). External sources such as sunlight and other forms of radiation can generate endogenous ROS, which can lead to a number of diseases (Del Rio *et al*.,2005). ROS can also be formed in food through lipid oxidation and photosensitizers exposed to light (Fang *et al*, 2002).

2-2-1-2 Reactive Oxygen Species – Classification

Reactive Oxygen Species are constantly being generated in the body, as a result of the normal metabolic processes. Mitochondria, which consume more than 90% of the oxygen in aerobic living organisms, are the main source of ROS and free radicals. Approximately 1% to 5% of the oxygen consumed by mitochondria is reduced and converted to these ROS (Mc Cord, 2000). ROS can be classified into oxygen- centered radicals such as superoxide anion (O_2^-), hydroxyl radical ($OH^\bullet$), alkoxyl radical ($RO^\bullet$), peroxyl radical ($ROO^\bullet$) and oxygen-centered non-radical derivatives such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Other common reactive species are nitrogen species such as nitric oxide ($NO^\bullet$), nitric dioxide ($NO^\bullet_2$) and poroxynitrite ($OONO^-$) (Bartosz, 2003; Lee and Min, 2004).

Reactive Oxygen Species cause lipid oxidation, protein oxidation, DNA strand breakage and modulation of gene expression. They are involved in many disease such as atherosclerosis, cancer, stroke, asthma, arthritis and other age related disease (Mc Cord 2000; Dimitrios, 2006; Niki, 2010). Some of the biological damage of macromolecules caused by ROS in the human body is outlined in Figure (1)

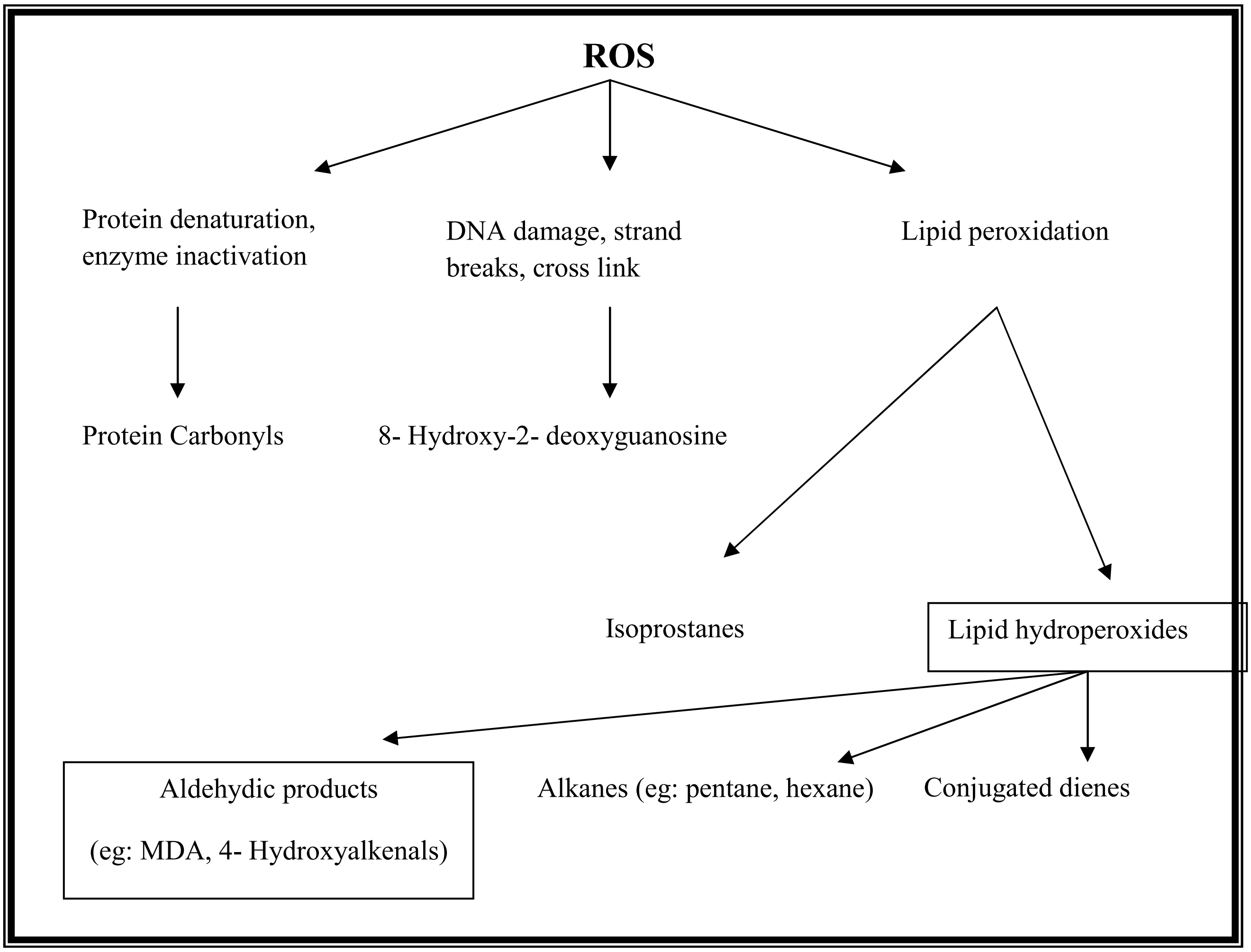


Fig (1): Damage of macromolecules caused by reactive oxygen species (ROS) and breakdown products of these molecules that are commonly measured (Lee *et al*., 2004).

2-2-1-3 Reactive Oxygen Species – Enzymatic formation

Lipoxygenases (LOXs), are found in plants and animals. LOXs are large monomeric protein with non-heme iron cofactor containing dioxygenases (COX-1, COX-2) catalyzing the oxidation of the PUFA forming hydroperoxides (Brash, 1999). Substrates for LOXs in plants are linoleic acid (18:2) and linolenic acid (18:3) and in animals are arachidonic acid (20:4) and decosahexenoic acid (22:6). In mammals, LOXs need free forms of PUFA, which are not present in healthy tissue. PUFA can be released from glycerides by membrane bound phospholipase. Free PUFA are oxidized to form lipid hydroperoxide (Brash, 1999). Lipoxygenase with Fe^{+2} ion exists in the inactivated status.

Once Fe^{+2} is oxidized to Fe^{+3}, Lipoxygenase can convert PUFA into hydroperoxides (Spteller, 2001). LOXs has been identified within atherosclerotic lesions, which suggests that this enzyme may be involved in the in *vivo* lipid oxidation (Knight, 2000).

2-2-1-4 Reactive Oxygen Species -Mechanism

Superoxide anion ($O^{\bullet}_2$)

Superoxide anion and hydrogen peroxide are the main ROS causing the oxidation of cells and tissues. $O^{\bullet}_2$ itself is not a strong oxidant. It reacts with protons in water solution to form hydrogen peroxide (equation 1-3), which can serve as a substrate for the generation of hydroxyl radicals and singlet oxygen (Mc Cord, 2000; Antolovich *et al.*, 2002).

$2O^{\bullet}_2 + 2H^+ \rightarrow H_2O_2 + O_2$ equation (1)

$HO^{\bullet}_2 + O^{\bullet}_2 + H^+ \rightarrow H_2O_2 + O_2$ equation (2)

$2HO^{\bullet}_2 \rightarrow H_2O_2 + O_2$ equation (3)

The superoxide anion can react with nitric oxide (NO^o) and form peroxynitrite ($ONOO^o$). The conjugate acid of peroxynitrite, peroxynitrious acid, can further decompose to generate hydroxyl radical and nitric dioxide (equation 4) (Knight, 2000).

$ONO_2 + H^+ \rightarrow ONO_2\,H \rightarrow {}^{\bullet}OH + NO_2$ (equation 4)

Superoxide can reduce Fe^{+3} to Fe^{+2}. The last two reactions are known as Haber-Weiss reaction (equations 5-6).

Haber – Weiss $O^o_2 + Fe^{+3} \rightarrow O_2 + Fe^{+2}$ (equation 5)

$H_2O_2 + O^{\bullet}_2 \rightarrow O_2 + OH^- + OH^{\bullet}$ (equation 6)

Hydroxyl radical (${}^{\bullet}OH$)

Hydroxyl radical is a highly reactive free radical (diffusion rate – limited) that can react with most living organisms. It react with lipids, polypeptides, proteins, and DNA (Mc Cord, 2000;Apak *et al*.,2007).A hydroxyl radical is highly electrophilic and can abstract electrons from proteins and PUFA, resulting in the formation of carbon - centered free radicals ($R^•$) (equation 7). The carbon centered free radical can undergo further reactions, such as reaction with oxygen, to give peroxyl radical ($ROO^•$) and alkoxy radical ($RO^•$) (equation 8-10) (Nawar, 1996)

$RH + {}^•OH \rightarrow R^• H_2O$ (equation 7)

$RO^• + O_2 \rightarrow ROO^•$ (equation 8)

$ROOH + Fe^{+3} \rightarrow ROO^• + Fe^{+2} + H^+$ (equation 9)

$ROOH + Fe^{+2} \rightarrow RO^• + Fe^{+3} + {}^-OH$ (equation 10)

Hydroxyl radicals can damage cell membranes and lipoproteins (the particles carrying cholesterol and triglycerides in the blood stream) by lipid peroxidation, that occurs by a radical chain reaction (once started, it spreads rapidly and affects a great number of lipid molecules) (Mc Cord, 2000; Del Rio *et al*., 2005). For example, PUFA's are mainly located in glycerolipids and phospholipids of cell membranes; therefore, cell membranes are easily oxidized and lose their functionality. Proteins may also be damaged by ROS, leading to structural changes and loss of enzyme activity. Oxidative damage to DNA also can cause DNA mutation, single strand breaks and chromosomal shortening (Del Rio *et al*., 2005).

Hydrogen peroxide (H_2O_2)

H_2O_2 can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Monomeric oxidases located in the outer mitochondrial membrane such as amino acid oxidase and xanthine oxidase also produce H_2O_2 from superoxide anion (Apak *et al.*, 2007). H_2O_2 is highly diffusible and crosses the plasma membrane easily. Once generated H_2O_2 is metabolized by catalase or glutathione peroxidase to generate water and oxygen. Although H_2O_2 is the least reactive molecule among ROS it is very damaging because it can be converted to hydroxyl radical (Del Rio *et al.*,2005). In the presence of metals such as Fe^{+2} or Cu^{+2}, H_2O_2 can generate hydroxyl radical through Fenton type reactions in which Fe^{+2} is oxidized to Fe^{+3} and H_2O_2 is converted to ºOH and ^{-}OH (equation 11).

Fenton $H_2O_2 + Fe^{+2} \rightarrow {}^{-}OH + {}^{\bullet}OH + Fe^{+3}$ (equation 11)

Singlet oxygen

Singlet oxygen is a non radical oxygen species. It can be generated from H_2O_2 during interaction with hypochloride (OCl^{-}) in cells and tissues (equation 12) (Fang *et al.*,2002). It can stimulate formation of nitric oxide (NO) and nitric dioxide (ONOO) (equations 13-15). Singlet oxygen is a mild toxin for mammalian tissue in comparison with other ROS. However, singlet oxygen has been known to be associated with oxidation of PUFA to initiate lipid peroxidation (Knight, 2000; Antolovich *et al.*, 2002).

$O^{\bullet}_2 + O^{\bullet}_2 + 2H^{+} \rightarrow H_2O_2 + {}^{1}O_2$ (equation 12)

${}^{-}OCL + H_2O_2 \rightarrow H_2O + CL^{-} + {}^{1}O_2$ (equation 13)

$ONOO^{-} + H_2O_2 \rightarrow NO_2 + H_2O + {}^{1}O_2$ (equation 14)

$O^{o}_2 + H_2O_2 \rightarrow {}^{-}OH + {}^{o}OH + {}^{1}O_2$ (equation 15)

Peroxyl and alkoxyl radicals

Peroxyl radicals (ROO^{o}) are formed by a direct reaction of oxygen with alkyl radicals (R^{o}), for example, the reaction between lipid radicals and oxygen. Decomposition of alkyl peroxides (ROOH) also results in peroxyl (ROO^{o}) and alkoxyl (RO^{o}) radicals. Peroxyl and alkoxyl radicals are good oxidizing agents which are involved in the propagation stage of lipid peroxidation (Nawar, 1996; Lee and Min, 2004).

Reactive nitrogen species

- Nitric Oxide and Nitric dioxide

Nitric oxide (NO^{o}) is formed *in vivo* from L-arginine by Nirtic oxide synthase. NO^{o} itself is not a very reactive free radical, but over production of NO can lead to protein oxidation in ischemia / reperfusion and neurodegenerative and chronic inflammatory diseases (Apak *et al*., 2007). Nitric dioxide (NO^{o}_2) is formed from the reaction of peroxyl radical and NO, polluted air and smoking (Noguchi and Niki, 1998).

- Peroxynitrite

Peroxynitrite can be generated by the reaction of NO^{o} and superoxide anion (equation 16). Peroxynitrite is acytotoxic species and causes tissue injury and oxidizes LDL. It can cause direct protein oxidation and DNA base oxidation (Apak *et al*., 2007).

$$O^{\bullet}_2 + NO^{\bullet} \rightarrow ONOO^- \qquad \text{(equation 16)}$$

2-2-2 Reactive Oxygen Species and lipid peroxidation in cell membrans

Del Rio *et al*., (2005) and Niki (2010) detailed that ROS with their differing reactivities not only generate local oxidizing effects, but also provide damaging pathological repercussions at regions away from the original area of production. In addition, ROS have the ability to initiate and propagate free radical chain reactions that can

damage many biological substances (Del Rio *et al*.2005; Mc Cord, 2000). Even though hydroxyl radicals have a half-life of nanoseconds it forms toxic products that can migrate to great distances to cause lipid peroxidation to vulenerable PUFAs in plasma membranes and organelles (Mc Cord, 2000). Cell membranes are phospholipid bilayers and protein, which are the direct targets of lipid oxidation. Lipid oxidation is a self-propagation reaction that leads to generation of lipid radicals and lipid peroxide (Lee and Min, 2004; Del Rio *et al*., 2005). As lipid oxidation of cell membranes increases, the polarity of lipid phase and formation of protein oligomers increase but molecular mobility of lipids, number of SH groups and resistance to thermodenaturation decrease (Mc Cord, 2000).

The resultant PUFAs bond modification forms conjugated dienes, which react with more O_2 to produce peroxyl radicals, lipid radicals ($L^{\bullet}$) and lipid hydroperoxide (LOOH) all of which can continue chain propagation (Ahotupa and Vasankari, 1999; Lapenna *et al*., 2001). However, the discontinuance of propagation may be caused by antioxidant such as α-tocopherol and natural phenolic compounds, which scavenge peroxyl radicals in the presence of vitamin C (Fang *et al*., 2004; Del Rio *et al*., 2005). The metabolites formed from lipid peroxidation are alkanels and aldehydic products such as malondialdehyde (MDA). Intracellular and plasma membrane injury can be due to MDA's ability to induce cross-linking of structural proteins, enzymes, DNA, RNA, and phospholipids. In addition, MDA changes membrane permeability, ion transport, alters LDL degradation, causes loss of PUFAs and is mutagenic, cytotoxic and carcinogenic (Knight, 2000; Stadman and Levine, 2003; Del Rio *et al*., 2005). An increase in MDA levels implies an increased level of oxygen radicals, which may be caused by an increased production of ROS and or decreased antioxidant reserve. An increased formation of ROS may be through sources such as xanthine – xanthine oxidase system, auto-oxidation of catecholamines and

homocysteine, prostaglandin synthesis and polymorphonuclear leukocyte stimulation (Fang *et al*., 2004)

2-2-3 Lipid peroxidation in foods

Lipid peroxidation can cause rancidity of food products such as fats and oils, which affect odor, taste ad nutritional value of these products. Lipid peroxidation proceeds, (Antolovich *et al*., 2002) *via* three different pathways:

(1) Non-enzymatic free radical-mediated chain reaction

(2) Non-enzymatic, non-radical photo-oxidation and

(3) Enzymatic reaction.

An example of pathways (2) is the stoichiometric oxidation of oleic acid by singlet oxygen to produce allylic hydroperoxides *via* addition of oxygen at either end of the double bond. The singlet oxygen is produced by sensitizers such as myoglobin or chlorophyll. Pathway (3) involves the action of lipoxygenases on various substrates.

Pathway (1) is the classical free radical route that leads to initiation of rapidly progressing, destructive chain reactions. The essential features of oxidation *via* a free radical-mediated chain reaction are initiation, propagation, branching and termination steps (Nawar, 1996). The process may be initiated by the action of external agents such as heat, light or ionizing radiation or by chemical initiation involving metal ions or metalloproteins (Nawar, 1996; Fang *et al*., 2002). The oxidation of the lipid involves hydrogen abstraction to form a lipid radical ($L^{\bullet}$) (equation 17), which can rapidly react with oxygen to form a lipid peroxyl radical ($LOO^{\bullet}$) (equation 18). The peroxyl radical propagates the chain

reaction by abstracting hydrogen from another lipid (equation 19), usually the rate-limiting step in lipid peroxidation. (Antolovich *et al*, 2002)

Initiation …………………. $R^{\bullet} + LH \rightarrow RH + L^{\bullet}$ (equation 17)

Propagation
$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ (equation 18)
$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ (equation 19)

The breakdown of lipid hydroperoxides (LOOH) often involves transition metal ion catalysis(M^{n+}), in reactions analogous to that with hydrogen peroxide, yielding lipid peroxyl and lipid alkoxyl radicals: (equation 20-23)

Branching
$LOOH \rightarrow LO^{\bullet} + HO^{\bullet}$ (equation 20)
$2\ LOOH \rightarrow LOO^{\bullet} + Lo^{\bullet} + H_2O$ (equation 21)
$LOOH + M^{n+} + H^{+} \rightarrow LO^{\bullet} + M^{(n+1)+} + H_2O$ (equation 22)
$LOOH + M^{(n+1)+} + OH^{-} \rightarrow LOO^{\bullet} + M^{n+} + H_2O$ (equation 23)

Termination reactions involve the combination of radicals to form non-radical products. (equation 24-26)

Termination
$LO^{\bullet} + LO^{\bullet} \rightarrow$ non radical products (equation 24)
$LOO^{\bullet} + LOO^{\bullet} \rightarrow$ non radical products (equation 25)
$LO^{\bullet} + LOO^{\bullet} \rightarrow$ non radical products (equation 26)

2-3 Antioxidants

2-3-1 Antioxidants in living organisms

Antioxidant defense systems have evolved in living organisms to counteract the potential damage caused by the generation of ROS. Antioxidants can be classified into two major categories: enzymatic, including superoxide dismutase, glutathione peroxidase, and catalase, and non-enzymatic, including glutathione and dietary antioxidants such as vitamin E, vitamin C, β-carotene and phytochemical compounds (Dimitrios, 2006;

Whitney and Rolfes, 2008). Superoxide dismutase, which catalyzes the dismutation of the superoxide anion to hydrogen peroxide and oxygen, exists in three forms: manganese – containing superoxide dismutase, is found in mitochondria and copper and zinc containing superoxide dismutase, is found in the cytosol as well as on cell surfaces (Finaud *et al*., 2006). The third form, iron containing superoxide dismutase, is found only in bacteria (Urso and Clarkson, 2003).

Catalase and glutathione peroxidase catalyse the reduction of hydrogen peroxide to water, and glutathione peroxidase also converts hydroperoxide to alcohol (Powers and Lennon, 1999). The action to these enzymes depends on the minerals, selenium, copper, manganese and zinc. If the diet fails to provide adequate supplies of these minerals, this line of defense weakens (Whitney and Rolfes, 2008). The body also uses the antioxidants (vitamins E, C, and β-carotene). Vitamin E defends the body's lipids (cell membranes and lipoproteins) by efficiently stopping the free radical chain reaction. β - Carotene also acts as an antioxidant in lipid membranes. Vitamin C protects other tissues, such as the skin and fluid of the blood, against free-radical attacks. It may also restore oxidized vitamin E to its active state. (Apak *et al*., 2007).

2-3-2 Natural antioxidants in foods

Natural antioxidants have been traditionally divided into two classes: primary or chain-breaking antioxidant and secondary or preventive antioxidants (Antolovich *et al*., 2002)

Primary (chain-breaking) mechanisms are represented by:

$$L^{\bullet} + AH \rightarrow LH + A^{\bullet}$$
$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet}$$
$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$$

The primary antioxidant, AH, when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical ($L^{\bullet}$) or inhibit the propagation step by reacting with peroxyl ($LOO^{\bullet}$) or alkoxyl ($LO^{\bullet}$) radical. The antioxidant free radical ($A^{\bullet}$) may further interfere with chain – propagation reactions by forming peroxy antioxidant compounds:

$$A^{\bullet} + LOO^{\bullet} \rightarrow LOOA$$
$$A^{\bullet} \rightarrow LO^{\bullet} \rightarrow LOA$$

The activation energy of the above reactions increases with increasing A – H and L – H bond dissociation energy. Therefore, the efficiency of the antioxidant increases with decreasing A – H bond strength (Nawar, 1996; Antolovich *et al.*, 2002).On the other hand, secondary (preventive) antioxidants retard the rate of oxidation. For example, metal chelators (e.g., iron sequesterants) may inhibit Fenton-type reactions that produce hydroxyl radicals (Apak *et al.*, 2007):

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{-} + OH^{\bullet}$$

Primary antioxidants may occur naturally or they may be produced synthetically as in the case of BHT, BHA, TBHQ and the gallates. The synthetic antioxidants are widely used in the food industry (Nawar, 1996) and are included in the human diet (Antolovich *et al.*, 2002; Dimitrios, 2006). The use of naturally occurring antioxidants has been promoted because of concerns regarding the safety of synthetic antioxidants, with natural alternatives (e.g. plant phytochemicals) possessing primary and secondary antioxidants activity similar to or even higher than that of synthetic antioxidants (Roberfroid,2000;Pourmorad *et al.*,2006).Plant phytochemical (an example of dietary antioxidants) can exhibit their antioxidant activity in several ways (Apak *et al.*,2007):

(1) Radical scavenging activity toward either reactive species (ROS) such as $OH^{\bullet}$, ${}^{\bullet}O^{-}_{2}$, ${}^{1}O_2$ or toward lipid peroxidizing radicals such as $R^{\bullet}$, $RO^{\bullet}$ and $ROO^{\bullet}$; radical

scavenging action generally proceeds *via* hydrogen atom transfer or electron donation.

(2) Prevention of the transition metal-catalyzed production of reactive species (*via* Fenton type reactions) through metal chelation.

(3) Interaction with other antioxidants (such as cooperative actions), localization and mobility of the antioxidants at the microenvironment.

These actions play key roles in defending the body against oxidative stress.

2-3-3 Measuring total antioxidant capacity(TAC)

Different assays have been introduced to measure antioxidant capacity of foods and biological samples. The concept of antioxidant capacity first originated from chemistry and was later adapted to biology, medicine, epidemiology and nutrition (Bartosz, 2003; Niki, 2010). Antioxidants capacity refer to the number of moles of a given oxidant scavenged by the sample studied. The word *total* should refer to measure all the antioxidant present in a complex sample (although it usually excludes specific antioxidant enzymes). TAC is often used to evaluate health beneficial effects of antioxidants. It appears to have a biological meaning as a parameter measuring the resultant force counteracting undesired oxidation in the material studied. It may detect a possible synergism between known antioxidants and the contribution of unknown antioxidants (cooperative action of antioxidants) (Antolovich *et al.*, 2002; Niki, 2010). The TAC database for vegetables, fruits, beverages, spices, and other food stuffs has been measured by ORAC, TEAC, TRAP and FRAP methods to investigate the role of dietary antioxidants. Often, Trolox is used as a reference compound and the capacity is expressed as Trolox equivalent (Apake *et al.*, 2007; Bartosz, 2003). The TAC values have been

measured also for serum and plasma to assess the *in vivo* antioxidant and nutritional status. The capacity of antioxidants *in vivo* has been assessed from the change in TAC value after intake of the antioxidant or antioxidant-containing products (Zulueta *et al*., 2009; Niki, 2010).

Antioxidant capacity assays may be broadly classified as electron transfer (ET)- and hydrogen atom transfer (HAT)- based assays (Apak *et al*., 2007; Zulueta *et al*., 2009), though in some cases, these two mechanisms may not be differentiated with distinct boundaries. In fact, most non-enzymatic antioxidant activity (such as, scavenging of free radicals, inhibition of lipid peroxidation, etc.) is mediated by redox reactions (Apake *et al*., 2007). HAT-based assays measure the capability of an antioxidant to quench free radicals (generally peroxyl radicals) by H-atom donation. The HAT mechanism of antioxidant action in which the hydrogen atom ($H^•$) of a phenol (Ar-OH) is transferred to an $ROO^•$ radical can be summarized by the reaction:

$$ROO^• + AH/Ar\text{-}OH \rightarrow ROOH + A^• / Ar\,O^•$$

where the aryloxy radical ($ArO^•$) formed from the reaction of antioxidant phenol with peroxyl radical is stabilized by resonance. The AH and ArOH species denote the protected biomolecules and antioxidants, respectively. Effective phenolic antioxidants need to react faster than biomolecules with free radicals to protect the latter from oxidation (Apake *et al*., 2007; Zulueta *et al*., 2009).As an example of HAT-based assays, ORAC assay applies a competitive reaction scheme in which antioxidant and substrate kinetically compete for thermally generated peroxyl radicals through the decomposition of azo compound such as ABAP (Apke *et al*., 2007; Zulueta *et al*., 2009;Niki, 2010). On the other hand, the ET mechanism of antioxidant action is based on the reactions:

$$ROO^• + AH/ArOH \rightarrow ROO^- + AH^{•+} /ArOH^{•+}$$

$AH^{\bullet+}$ /ArOH $^{\bullet+}$ + H_2O ↔ $A^{\bullet}$ / $Aro^{\bullet}$ + H_3O^+
ROO^- +H_3O^+ ↔ ROOH + H_2O

The aryloxy radical ($Aro^{\bullet}$) is subsequently oxidized to the corresponding quinone (Ar = O). The more stabilized the aryloxy radical is, the easier will be the oxidation from ArOH to Ar = O due to reduced redox potential. In fact, in most ET-based assays, the antioxidant action is stimulated with a suitable redox-potential probe, i.e., the antioxidants react with a fluorescent or colored probe (oxidizing agent) instead of peroxyl radicals (Apake *et al.*, 2007). Spectrophotometric ET-based assays measure the capacity of antioxidant in the rediction of an oxidant, which changes colour when reduced. The degree of colour change (either an increase or decrease of absorbance at a given wavelength) is correlated to the concentration of antioxidants in the sample (Apak *et al.*, 2007; Niki, 2010). ABTS / TEAC (Trolox ® - equivalent antioxidant capacity) and DPPH are decolorization assays, whereas in Folin total phenols assay, FRAP and CUPRAC, there is an increase in absorbance at a prespecified wavelength as the antioxidant reacts with the chromogenic reagent. The basic chromophores used in Folin, ABTS / TEAC, FRAP, DPPH and CUPRAC assays are shown in Figure (2).

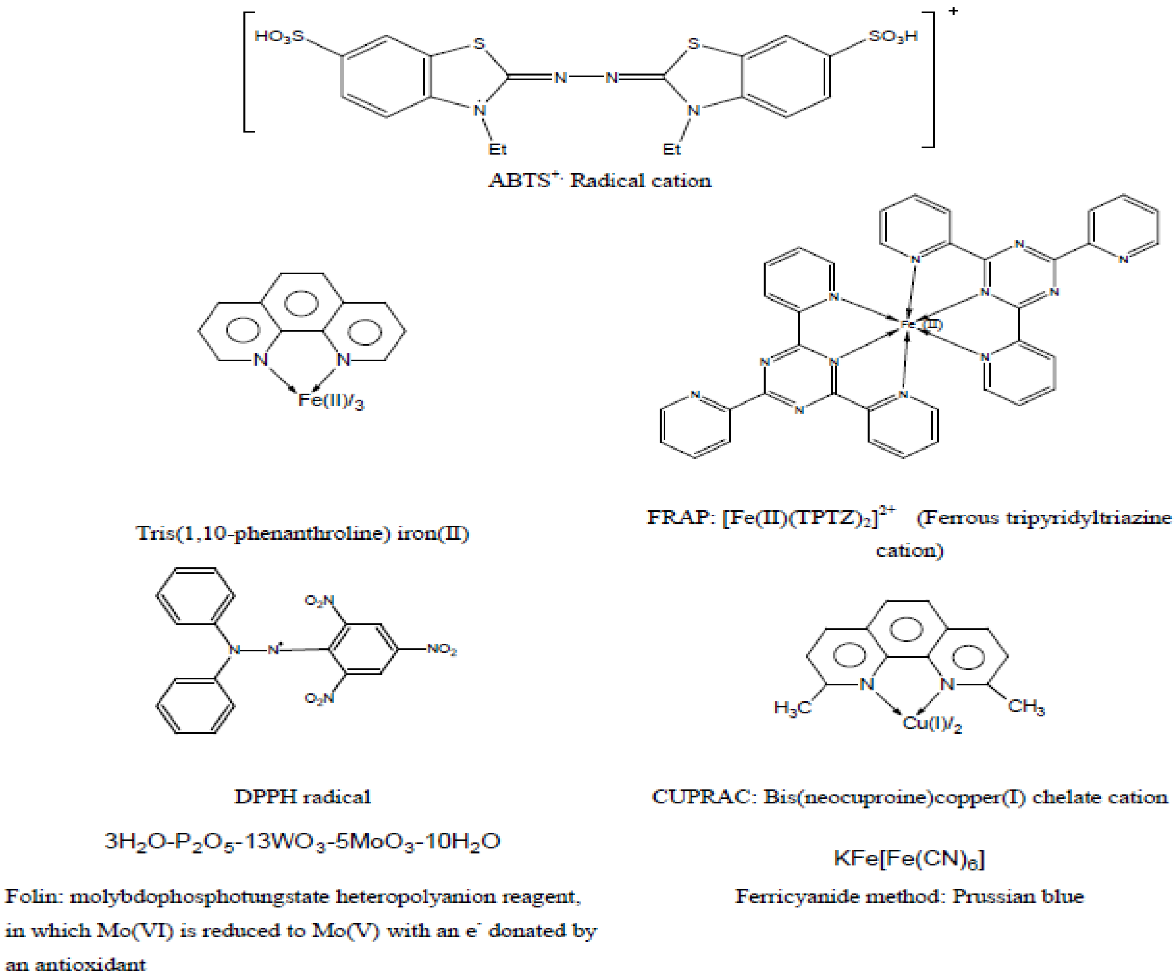


Figure (2): Basic chromophores used in TAC (total antioxidant capacity) assays (Apak *et al.,*2007)

2-3-4 Measuring lipid peroxidation

Of the many biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid oxidation gives rise to a number of secondary products. These products are mainly aldehydes (such as MDA), with the ability to exacerbate oxidative damage (Uchida, 2000, Del Rio *et al*., 2005). MDA is the principal and most studied product of PUFA peroxidation. Since the 1960 several methods have been developed to assess this molecule in order to quantify the level of oxidative stress *in vivo* (Lapenna *et al*., 2001; Niki, 2010).

The thiobarbituric acid reactive substances (TBARS) assay was proposed over 45 years ago and is now the most commonly used method to detect lipid oxidation (Antolovich *et al*., 2002; Apake *et al*., 2007). This procedure measures the MDA formed as the split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of a lipid substrate. It is postulated that the formation of MDA from fatty acids with two or more methylene interrupted double bonds, PUFA, occurs *via* the secondary oxidation of primary carbonyl compounds (non – 2 – enal). (Del Rio *et al*., 2005; Niki, 2010). The MDA is reacted with thiobarbituric acid (TBA) to form a pink pigment (TBARS) (Figure3) that is measured spectrophotometrically at its absorption maximum at 532- 535 nm (Lapenna *et al*., 2001; Antolovich *et al*., 2002).

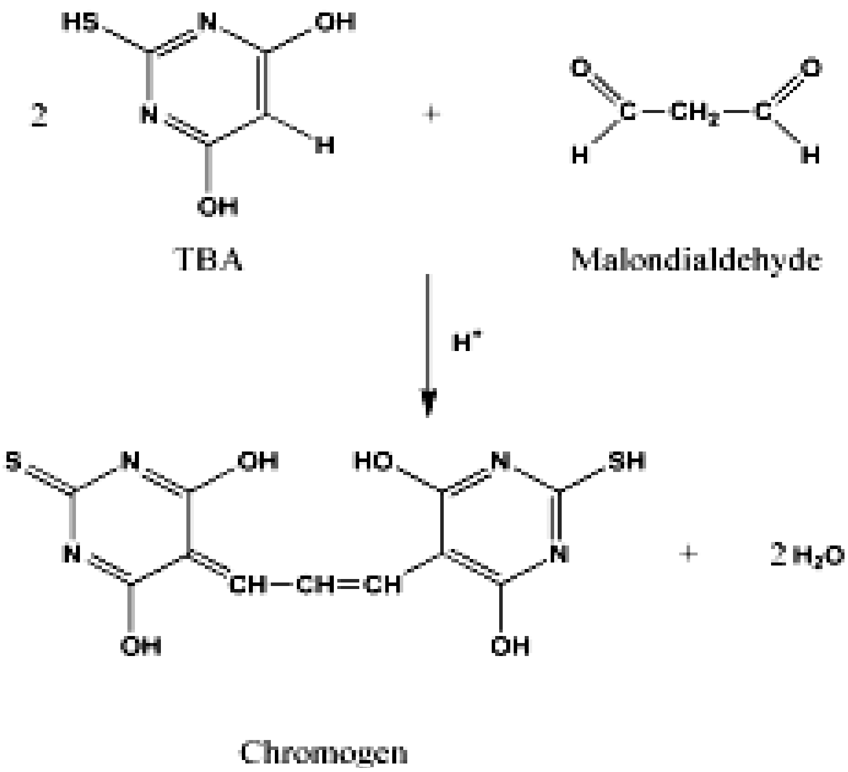


Figure (3): Chromophore formed by condensation of MDA with TBA. (Antolovich *et al*, 2002)

Numerous substrates have been used in the determination of TBARS, including tissue samples, linoleic acid and other fatty acids and LDL (Antolovich *et al*., 2002). The procedure involves two distinct steps: the substrate is oxidized with the addition of a transition metal ion such as copper or iron or a free radical source and then the extent of oxidation is determined by addition of TBA and spectrophotometric measurement of the product. Oxidation is inhibited by the addition of an antioxidant and therefore a reduction in the absorbance is seen. Results may be described in terms of percentage inhibition of the oxidation. The TBARS procedure is widely used even though the reaction is not very specific and reaction conditions have a significant effect on colour development (Del Rio *et al*., 2005, Niki, 2010). Selectivity of the TBARS procedure is improved by the use of HPLC to characterize the individual species, but this still does not identify the source of MDA in samples or eliminate the possibility of a compound with similar spectral properties coeluting (Antolovich *et al*., 2002; Niki, 2010).

3- Wild Edible Plants in the Human Diet

3-1 Importance of wild edible plants

Although the use of wild and traditional food plants has been influenced by the economic attraction of food and non-economic crops, they are of potential importance in family food security since people culturally accept them and can use them in a variety of ways (Abu-Rajouh and Takruri, 2000). The study of the nutrient composition of such plants has become common in different parts of the world. Many of these plants are nutritionally important because of high vitamin, mineral, fiber, protein and antioxidant contents (Abu-Jadayil *et al*., 1999). Furthermore, some of these wild plants are being cultivated or have become available not only in local markets but have also been introduced to markets and cuisines of industrialized countries (Roberfroid, 2000).

In addition, some of these plants have been reported to have various therapeutic uses. Therefore, the identification of these plants as well as the study of their chemical composition, nutritive value, methods used in preparation and ways they are being consumed have become necessary in many regions. Such information would be helpful for use in nutritional and food planning (Al-Eiswi and Takruri, 1989; Tukan *et al*., 1998).

3-2 Wild edible plants in the Jordanian diet

The use of wild edible plants in human diet is increasing all over the world (Abu-Jadayil *et al*.,1999). In Jordan, large numbers of wild edible plants have been reported to be distributed throughout the country and consumed in various ways (Al-Eiswi and Takruri, 1989; Tukan *et al*., 2008).

Some of these plants can be consumed raw and some of them without any preliminary preparation other than trimming and cleaning (e.g. milk thistle stems and seeds). They are usually picked when in season and eaten as snacks particularly during picnics, shepherding or travelling in the area (Tukan *et al*., 1998). Such plants can be considered to be dietary fiber, vitamin and mineral-rich snacks in comparison with some modern empty-calorie foods. An example of such plants eaten raw are: dill, fennel, garden rocket, hawthorn, mustard, Roman nettle, milk thistle, wild lettuce, wild mint, wild pea, winged peas, and ziziphus (Tukan *et al*.,1998).

Tukan and colleagues (1998), conducted a comprehensive study, about 56 wild edible plants used in the Jordanian diet, to determine the plant parts used, the detailed methods of their preparation and preservation as well as their uses. They found that the plants used are either eaten raw, cooked by boiling in water, frying in fat or baking to be served as dishes such as stew, turnovers, stuffed and rolled vegetable foods or as cold or

hot drink for certain occasions and seasons. Also, they concluded that these plants should be further investigated for detailed nutrient composition and their potential contribution to nutrient intake of Jordanians.

3-3 Edible plant seeds

3-3-1 Overview of seeds:

Many food crops are grown for their whole fruits and include wheat, corn, rice, safflower, soybean, rapeseed, peanut, sunflower, cotton and canola. However, some food crops are only grown for specific parts of their fruit such as the mesocarp (flesh), and their seeds are discarded as waste by products. These types of crops are primarily harvested for Juice and Juice concentrate, wine, jam, soup, and salad production, and billions of tons of their seeds are discarded each year either as cheap animal feed or simply thrown away at a cost (Gorinstein *et al*., 2003;Cintra *et al*., 2006).

Seed components can be separated into two parts: oil and flour or cake. The flour remains following oil extraction, which is performed by many methods including solvent extraction and cold-pressing (Tuberoso *et al*., 2007). Seeds have very different and complex chemical compositions that are nutritionally grouped as macronutrients, micronutrients and other components. Other components include phytochemicals such as phenolic antioxidants that have demonstrated potential beneficial health properties (Moon et al., 2001; Bail *et al*., 2008; Garjini *et al*., 2009). Seeds oils also have other properties that include oxidative stability and color. These components and properties of seeds are valuable and need to be examined and reported which may ultimately lead to increased crop values and increased farm-gate profits for growers and processors (Barreto *et al*.,2003; Parry *et al*., 2008; Visavadiya and Marasimhacharrya, 2008; Shaker *et al*.,2010).

3-3-2 Extraction of seeds oil

Oils are conventionally extracted from ground seed meal using organic solvents such as hexane with heat, followed by solvent evaporation (Barreto *et al*., 2003; Subramaniam *et al*., 2008). Cold-pressing is a method that does not use organic solvents or heat to extract seed oil. A typical cold-press machine is screw driven and removes oil by applying grinding pressure to seeds. The residual remaining after extraction is the cake or flour (Parry, *et al*., 2008, Bail *et al*., 2008). Another type of cold-pressing applies pressure directly to seeds in a barrel with slits down the side that allow oil to run out (Parker *et al*.,2003).In this process, neither the raw materials nor the oil are exposed to higher temperatures in accordance with the Codex Alimentarius for cold-pressed oils. The Codex Alimentarius does not allow application of heat in the course of any production step of cold-pressed oils (FAO/WHO, 2005).

Cold-pressing is belived to be a better technique for retaining beneficial value-added components (more phytochemicals) in seed oils that might be lost by evaporation or chemically modified using conventional solvent extracting methods (Parker *et al*., 2003; Tuberoso et al., 2007; Parry et al., 2006).The shelf life of seeds oil may vary depending on the type of oil as well as the storage conditions. For example ,flax and grape seed oil have a shorter shelf life than canola or sunflower due to the large amount of polyunsaturated fatty acids present (Vujasinovic *et al*.,2010).

3-3-3 Chemical components

3-3-3-1 Macronutrients

The macronutrients in cold-pressed seeds include protein, carbohydrates, fats, and fiber. The oil from cold-pressed seeds mainly contains lipophilic compounds while the

flour is comprised of both hydrophilic and hydrophobic compounds. Cold-pressed seed oils are predominantly triglycerides which are 3 fatty acids esterified to a glycerol backbone, while flours are comprised of a mixture of the macronutrients. (Moon *et al*., 2001; Tuberoso *et al*., 2007; Parry *et al*., 2008). Fatty acids are vital components of all human diets not simply as an energy source, but they are required by all cells of the body in the form of phospholipids for structural membrane integrity (Whiteny and Rolfes, 2008). Seed oils are comprised of fatty acids that differ in their carbon chain lengths; however, they are mainly comprised of fatty acids with 16, 18 and 20 carbons. Two fatty acids, (Linoleic acid (18:2 n-6) and α-linolenic acid (18: 3 n-3)) found in seed oils are essential to human diets because they cannot by synthesized *de novo*; therefore, they must be obtained from exogenous sources (Cintra *et al*.,2006; Parry *et al*., 2006; Whitney and Rolfes, 2008). These essential fatty acids are required by the brain and nervous system and for normal growth and vision development in infants (Simopoulos, 2002). Aside from being nutritionally essential, linoleic and linolenic acids have been considered cholesterol lowering fatty acids, and LDL is the primary cholesterol that is decreased by their effect (Whitney and Rolfes, 2008). Oleic acid (18: 1 n-9) is another important fatty acid found in seed oils. It is the primary fatty acid found in olive oil (68-82%), canola oil (63%) and sesame oil (44%) (Baba *et al*., 2000) and is also found in considerable amounts in many other oils. Diets high in oleic acids have been experimentally demonstrated to lower total cholesterol – primarily LDL cholesterol. Also, a meta analysis of several previous studies revealed that there was no significant difference between diets containing high levels of oleic acid and diets with high levels of linoleic acid in their effectiveness to lower total cholesterol and LDL when experimentally replaced for saturated fat in equal amounts (Simopoulos, 2002). Furthermore, LDL composed of high levels of oleic acid have shown

a decreased susceptibility to oxidation compared to LDL containing high concentrations of PUFA (Micallef *et al*.,2009).

3-3-3-2 Micronutrients

Micronutrients found in seeds include vitamins, provitamins, and minerals. Fat soluble vitamins including vitamins E, K and provitamin A are primarily found in the oil. The seed flour also contains the fat- soluble vitamins at lower concentrations than in the oil, but also contains water soluble vitamins including the vitamin B complex if present in the seed (Moon *et al*., 2001;Cintra *et al*., 2006; Parry *et al*.,2008).

Vitamin E is comprised of eight different forms that are further classified into 2 groups of 4 called tocopherols and tocotrienols. The tocopherols have saturated side chains and the tocotrienols are unsaturated with three conjugated double bonds on the side chain. The classes of both are differentiated by substitutions of methyl groups on the phenol ring and are named α, β, δ and γ tocopherol or tocotrienol. The 4 tocopherols have vitamin E activity while α-tocotreinol demonstrates appreciable activity among the tocotrienols (Ha *et al*.,2005). Vitamin E primarily functions to maintain stability of membranes by preventing initial oxidation and stopping the cascading oxidation of PUFA's by lipid peroxyl radicals (Whitney and Rolfes, 2008).

Vitamin A, as retinol, is not found in seed oils, however, precursors of vitamin A including β-carotene and α-carotene, are prevalent. When hydrolyzed, β-carotene provides 2 retinol molecules while α and γ– carotene can provide one (Nawar, 1996; Parry, *et al*., 2006).Vitamin A performs many important functions in the human body. Other carotenoids also found in seed oils that include lutein, cryptoxanthin and zeaxanthin that may posses antioxidant abilities (Bail *et al*., 2008).

3-3-3-3 Antioxidants

Natural antioxidant compounds found in edible seed oils and flours include the previously discussed vitamin E and carotenoids but also include phenolic acid and polyphenolic acids. The phytochemical compounds (mainly phenolic compounds) present in these seeds are a very important class of minor constituents and they are related to the stability of the oil as well as its biological properties (Besbes *et al*., 2004; Dimitrios, 2006). Individual phenolic compounds and foods containing phenolic compounds have shown strong abilities to significantly extended the presence of vitamin C when exposed to ascorbic acid oxidase (Pourmorad *et al*., 2006) and individual phenolic compounds have shown to be very free effective radical scavengers when compared to trolox, a water soluble vitamin E analog (Noguchi and Niki, 1998). The polypehnol content differs from one oil to another oil. Wide ranges have been reported (50-1000 mg/kg) (Dimitrios, 2006).

Olive oil has many phenolic compounds, mainly hydroxytyrosol and its derivatives, which were indicated to have a scavenging activity against superoxide anion and hydrogen peroxide and a capability to prevent the generation of reactive oxygen species. An *in vitro* inhibitory effect on platelet aggregation has indicated some mechanism by which olive oil phenols help to protect against various CVD disorders. Whereas their capacity to scavenge nitrogen reactive species such as peroxynitrile suggests a protective effect against nitration of tyrosine and DNA damage (Bianco et al., 2001; Blekas et al.,2002).

Both, Kamal-Eldin and colleagues (1994) and Shyu and Hwang (2002) studied the extraction and analysis of lignans in sesame seeds. They found that the antioxidant activity of sesame seed and sesame seed oil and the various healthful properties are attributed to the presence of lignans such as sesamin, sesamolin, sesaminol, sesangolin, 2 –episalatin and others. Also, they found that sesame oil is extremely resistant to oxidative rancidity

and this is due to the presence of other strong antioxidants (not Lignans) but chemically related compounds such as sesamol and sesamol dimer.

In addition to olive oil and sesame seeds, many oil seeds have been cold- pressed including marionberry, boysenberry, raspberry, blueberry, blackcaraway, blackcurrant carrot, cranberry and hemp seeds have been reported to contain antioxidants. The resulting oils were found to possess a remarkable radical scavenging activity and oxygen radical absorption capacity, when tested with the DPPH (1,1-diphenyl – 2- picryllhydrazyl) and ABTS cation (2, 2 azino-bis-3ethyl benzo-thiazoline – 6- sulfonic acid) radical-scavenging assays or the oxygen radical absorption capacity (ORAC) assay (Parry *et al*., 2005;2006).

Grape seed oil and flour was also studied for its total phenol content and antioxidant capacity in order to evaluate its nutritional potential use (Bail *et al*., 2008). Results of this study confirmed a high phenol content and antioxidant capacity of the oil extracts and flour. A previous investigation by Bagchi *et al* (2000) have demonstrated that grape seed oil and / or flour were more powerful radical scavengers than both vitamins C and E protecting against tobacco induced oxidative stress, skin damage from UV light and human liver cells *in vitro* against cytotoxicity among other protective qualities. The nature of the antioxidant is not yet known, but due to the mode of preparation these oils retain phenols present in the seed and they may have the potential for applications in promotion of health and prevention against oxidation damages mediated by radicals (Dimitrios, 2006).

Other cold pressed seed oils and / or flours currently investigated are date seed, argan, onion, cardamom, mullein, roasted pumpkin and milk thistle seeds (Besbes *et al*., 2004; Parry *et al*., 2006 , 2008).

4- Milk Thistle Plant (*Silybum marianum*, L. Gaertn)

4-1 Nomenclator and description

Milk thistle (*Silybum marianum*, L. Gaertn) is a wild plant from the *Compositeae* family. It is an annual plant, native to the Mediterranean area, including Jordan, and North Africa (Tukan *et al*., 1998; Fathi – Achahhlouei and Azadmard – Damirchi, 2009). It grows wild throughout Europe, some parts of the United States and Australia. It can be also cultivated (Barreto et al., 2003).

Milk thistle was described by Zohary (1986) to have the following characteristics:

- Height range from 25-200 cm or more
- Stem is stout, striate, simple or branched
- Leaves are white-veined and spotted along anastomozing veins, pinnatified into broad ovate-triangular sinuate-dentate spiny lobes; radical leaves often very large, tapering at base, cauline leaves auriculate clasping
- Heads are 6-10 cm in diameter. Spiny involucral bracts longer than florets. Florets purple to pale purple or white. Achenes obscurely striate, 6mn, pappus yellowish.
- Flowering date starts from March until May
- Each flower head produces approximately 100 seeds, and 10-50 heads are produced per plant, with an average of 3000 seeds per plant, 94% of them viable.
- The seeds show little to no dormancy requirements, and any dormancy length is affected by temperature and moisture. The seeds remain viable for 9 years or more.

Milk thistle plant and seeds are shown in figure 4 and 5, respectively.



Figure 4: Milk thistle plant (at the flowering stage).



Figure 5: Milk thistle plant seeds.

4-2 Uses and chemical composition

4-2-1 Dietary uses

Milk thistle is eaten in several places in the world at different maturity stages. In England, it is eaten with salads (Jacobs et al., 2002), but in USA, the seeds are ground up and used in beverages in place of coffee beans (Barreto *et al*., 2003).One of the important issues about milk thistle plant is that it may be accepted as a safe and well tolerated herbal product, since no health hazards or side effects are known (Jacobs *et al*., 2002; Shaker *et al*., 2010).

In Jordan, milk thistle is a common wild plant, which grows in many regions during the winter and spring seasons. People of the region, particularly in the rural areas, eat milk thistle. The stems are consumed raw or cooked, the flower heads are cooked, and the seeds are consumed raw or roasted (Abu-Rajouh and Takruri, 2000).

4-2-2 Medicinal uses

Recently, several scientific studies have investigated the chemical properties of milk thistle plant (Nyiredy *et al*., 2008; Shaker *et al*., 2010). Known for centuries as a "liver tonic", milk thistle is high in flavonolignas substances (especially silymarin) which are medicinally active. It has been reported to greatly improve the overall functioning of the liver, and is used for reducing cirrhosis of the liver, chronic liver inflammation, damage done to the liver through the intake of alcohol and other intoxicants, as well as gallbladder disease (Barreto *et al*., 2003; Kvansicka *et al*., 2003; Shaker *et al*., 2010). Early laboratory studies conducted by Katiyar (2005) and Ramasamy and Agarwal (2008) reported that Silymarin and other active substances in milk thistle seeds may have anticancer effects. These substances appear to stop cancer cells from dividing and reproducing, shorten their

life span, and reduce blood supply to tumors. As mentioned above, the consumption of milk thistle seed products has increased. In 2005, sales of MT products reached about 8.3 million dollars and were placed in top ten dietary supplements sold in USA that year (Subramaniam *et al*., 2008).

4-2-3 Chemical composition of seeds

4-2-3-1 Nutrient composition

Studies regarding the proximate analysis of MT seeds are few, but data available in scientific references mention that MT seeds contain, a considerable content of fat, protein, ash and fiber. The proximate composition of MT seeds is shown in Table (2).

In Jordan, the first study on the MT seeds was conducted by Abu-Rajouh and Takruri (1996) to determine the protein quality and identify the fatty acids pattern of MT seeds. The researchers found that oil, crude fiber and protein content of the seeds were high. The protein quality is intermediate between soybean and wheat protein.

The oil has a high linoleic acid followed by oleic acid, palmitic acid and stearic acid contents and thus could be included among plant oils which have dietary and health applications. This result regarding fatty acids pattern is almost similar to the findings of other studies conducted on (MT) seed oil. Tables (3) summarize the fatty acids content in MT seed oil reported in different studies.

Another study in Jordan, by Abu-Jadayil *et al*., (1998) examined the bioavailability of some minerals in rats for selected plants of which, was MT seeds. They found that the total absorption of calcium was the highest in MT seeds compared to other plants in the study, while calcium utilization in femur was the lowest. On the other hand, zinc and iron total absorption was low in rats fed MT seeds.

Table (2): Proximate composition of milk thistle seeds.

Component (g/100g)	El-Tahawi and Ali (1987)[1]	Abu-Rajouh and Takruri (1996)	Abu-Jadayil et al., (1999)
Moisture	-[2]	5.04	5.77
Protein	18.50	16.70	19.12
Fat	-	26.40	26.25
N.F.E (nitrogen free extract)	-	22.67	24.33
Ash	-	4.20	4.88
Fiber	-	25.00	25.42
Minerals(mg/100g)			
Ca	1361	-	1614.98
Fe	4.3	-	9.79
Zn	2.6	-	6.82

(1) *Adapted* from: Abu-Rajouh and Takruri,1996

(2) ND : not detected

Table (3) Fatty acids content in milk thistle seed oil as reported by different studies.

Fatty acid (Composition (%)		Kaezmarek andMyrugasiew-iez (1975)[1]	Hamid et al., (1983)[1]	Khan et al., (1985)[1]	Abu-Rajouh and Takruri (1996)	Parry et al., (2006)
C 14:0	Myristic acid	ND	ND	ND	ND	0.10
C 16:0	Palmitic acid	7.40	9.73	9.70	9.09	8.90
C 16:1	Palmitoleic acid	ND	ND	ND	ND	0.10
C 18:0	Stearic acid	5.03	7.07	7.10	6.15	4.80
C 18:1	Oleic acid	20.87	36.45	37.0	28.35	23.80
C 18:2	Linoleic acid	58.89	42.11.	42.0	51.46	60.8
C 18:3	Linolenic acid	1.44	ND	ND	0.18	0.20
C 20:0	Arachidic acid	3.33	3.30	3.30	3.74	ND
C 20:1	Eicosenoic acid	ND	ND	ND	ND	1.20
C 22:0	Behenic acid	3.04	3.04	3.00	1.00	ND
Total SAT		18.8	23.14	23.10	19.98	13.80
Total MUFA		20.90	36.45	37.00	28.35	25.10
Total PUFA		60.33	42.11	42.00	51.64	61.10

ND: not detected

(1)*Adapted* from: Abu-Rajouh and Takruri, 1996

Regarding caroteniods, low levels were detected in MT seed oils. Zeaxanthin, the pigment that gives corn its characteristic yellow color, was the primary carotenoids compound present (1.31 mg/kg), while the total carotenoids concentration were (2.30 μmol/kg).This level was comparable with those observed in cold pressed onion and cardamom seed oils (1.05 -3.00 μmol/kg). On the other hand, the cold-pressed roasted pumpkin seed oil had the highest total carotenoids concentration (70.6 μmol/kg) followed by the parsley (40.3 μmol/kg) and mullein seed oils (15.8 μmol/kg) when compared with MT seed oil (Parry et al.,2005, 2006). Also, Parry *et al*. (2006) and Fathi-Achachlouei and Azadmard-Damirchi (2009) reported a significant levels of tocopherols, mainly α-tocopherol, in MT seed oils. The α, β, γ and δ tocopherols ranged from 156 to 465, 10 to 51, 9 to 35, 7 to 80 mg/kg oil, respectively and the total tocopherols ranged from 225-632 μmol/kg oil.

4-2-3-2 Polyphenolic compounds

Milk thistle is a plant rich in polyphenolic compounds. Silymarin (SM), an extract from the seeds flour of MT usually contains 50-70% flavonolignans (silibinin, silybin and its diastereoisomers, isosilybin, silydianine and silychrsitin), flavonoid taxifolin and other minority compounds (Nyiredy *et al*., 2008; Shaker *et al*., 2010). Silibinin is the major bioactive component present in silymarin. It has metabolic and cell-regulation effects (like membrane-stabilizing and antioxidant), which have been considered to be major hepatoprotective mechanisms (Kvasnicka et al., 2003; Shaker et al., 2010). In addition, several lines of evidence point to a direct role for silymarin in the treatment of different types of cancer, as a direct anticarcinogenic agent and as an adjuvant in chemotherapy. Ramasamy and Agarwal (2008) and Shaker (2010) reported that silibinin is a promising new treatment for cancer.There are some data indicating the ability of silymarin to modulate and positively affect lipoprotein metabolism. The hypolipidaemic effects of silymarin and its polyphenolic fraction are manifested by a decrease in cholesterol levels in

the liver and plasma in rats fed on a high-cholesterol or high-sucrose diet (Skottova *et al*., 2004). Sobolova *et al*., (2006) have recently demonstrated that these changes are due to the fact that silymarin and its polyphenolic fraction inhibit the absorption of dietary cholesterol from the intestine. In a recent research conducted by Parry and colleagues (2008), the antioxidant properties and antiproliferative capacity of selected cold-pressed seed flours of which, MT seeds flour, was studied. The results of the study reported that the cold-pressed MT seeds flour is very rich in natural antioxidant components due to presence of high total phenolic content (25.5 mg gallic acid equivalent / g flour) (GAE mg/g) and thus, may serve as an edible source of antioxidant. Also, it contained on antiproliferative components. The antiproliferative effect of the MT seed-flour extract against HT-29 human colon cancer cells is consistent with the results of previous investigations on specific polyphenolic compounds, including silymarin in MT seeds.

Materials and Methods

1. Collection and Preparation of Milk Thistle seeds:

MT seeds were obtained by threshing the dried mature flowers which were collected from different rural areas of Jordan during the 2010 season (April –May). The seeds were dried under the sun for a few days, cleaned from impurities by using gradated sieves (different mesh sizes) and stored in airtight glass containers until oil extraction.

2. Proximate analysis of milk thistle seed

Proximate analysis of MT seeds used in the study was determined and included analysis for moisture, crude fat, crude protein, ash, crude fiber and soluble carbohydrate content according to the official methods of the Association of Official Analytical Chemists (AOAC, 2000). Procedures for analysis were performed as follow:

2.1. Moisture

Moisture was determined using the oven-drying method. A 3g homogenous sample was dried at a temperature of 105°C until constant weight was obtained. Weight difference was taken as the seed moisture content. All other analyses were carried out on a dry matter basis.

2.2. Crude protein

The macro-Kjeldahl method used to determine crude protein content. In this method, a sample (0.5g) was digested using hot sulphuric acid (98%) in the presence of a catalyst to break down the organic matter and reduce nitrogen to ammonia. Reduced ammonia was then librated by boiling with (50%) sodium hydroxide solution and the produced steam was distilled into boric acid (4%) to form ammonium acid (0.1 N) solution and the protein content was calculated by multiplying the nitrogen content by 6.25.

2.3. Crude fat

Crude fat was determined using Soxhlet extraction method as described by the AOAC, (2000). Petroleum ether was continuously vapourized and allowed to pass through the seed sample extracting the ether-soluble matter. After 8 hours, the extract was collected in another container and the remaining fat was dried and weighted.

2.4. Ash

Approximate 2-3g were placed in crucible and ignited in muffle furnace (Naber, Model D-2804, Bremen) the temperature was gradually brought to 550°C and left for 8-10 hours to burn-off all organic materials. The crucible with the ash remaining was removed from the furnace to a desicator using forceps and left to cool at room temperature then weighted. The weight of ash divided by the sample weight is the ash%.

2.5. Crude fiber

Crude fiber was determined by digestion of the fat-free seed sample in hot sulphuric acid solution (1.25%) and then in sodium hydroxide solution (1.25%). The sample was then dried, weighted and ashed for six hours at 550 C°. The difference in weight before and after ashing represents the crude fiber content.

2.6. Carbohydrates:

Nitrogen-free extract which represents carbohydrates was calculated by subtracting the sum of percentages of crude protein, crude fat, crude fiber and ash from 100%.

3. Milk Thistle Seeds Oil Extraction:

Cold- pressed MT seed oil was obtained by pressing raw- dried and cleaned seeds with a screw-press type unit (CLB-100, Sweden)[1], run by a 4-KW electric motor, capacity of 10-20kg/h, in a small-size facility for cold-pressed oils. The technological process of cold-

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[1] This process was done in the Green Fields Oils Factory for Cold Pressed Oil / Amman.

pressed MT seed oil included the following: the clean dry seeds were fed to the rotating screw inside a horizontal cylinder that is capped at one end. The screw forced the seeds through the cylinder with gradually increasing pressure. The seeds were heated by friction. Once the cap is removed, the oil escapes from the cylinder through small holes or slots and the press cake or meal emerges from the end of the cylinder as pellet form. The squeezed oil was collected in a stainless steel vessel (the oil temperature was directly measured at this step, reaching about 28°C). Turbid matter resulting from ruptured plant material of the seeds during pressing was removed by sedimentation or filtration at room temperature. The oil content of MTS ranged from 26 to 27.5 %. Filtered oil (Figure 6) was filled into dark glass bottles and kept refrigerated at 4°C. Because of this simple process, the oil maintains the natural composition with regard to the constituents and flavour.



Figure 6: Filtered milk thistle seed oil

4. Chemical Characterization of Milk Thistle Seed Oil:

The chemical characterization of cold-pressed MT seed oil used in the study were determined. These tests include oil quality analysis for acidity, peroxide value and oxidative stability index and instrumental analysis to determine the fatty acid profile, phytosterols profile, squalene, ∝-tocopherol, total phenolic content, radical ($DPPH^{\bullet}$) scavenging capacity and total antioxidant capacity.

4-1 Oil Quality Analysis:

4-1-1 Acidity[1]

The acidity of MT seed oil was determined as described by AOAC (2000). Five grams of oil sample were weighed in a 250 ml flask. The sample was dissolved in the 25 ml mixed neutral solvent (1:1 ethanol: diethyl ether). One ml of (1%) phenolphthalein indicator was added. Titration with 0.1N KOH was made under vigorous shaking until a faint pink color appears which lasts no more than 1 min. Acidity was calculated as % free fatty acids (FFA) expressed as linoleic acid according to the following equation.

$$\text{Acidity, FFA, as linoleic acid (\%)} = \frac{\text{ml KOH x N x mEq linoleic (0.28045)}}{\text{Wt}} \text{ x 100}$$

Where N is the normality of KOH and Wt is the weight of sample (g).

4-1-2 Peroxide Value [2]

The PV was determined as described by AOAC (2000). Five grams of MT seeds oil were weighted into a 250 ml glass stoppered conical flaks. 25 ml glacial acetic acid: chloroform solution (3: 2 V/V) and 1ml saturated KI were added. The flask was swirled until the sample was dissolved into the solution. The solution was allowed to stand in the

[1] This analysis was performed in the Chemical Division, Food Laboratory, JFDA.
[2] This analysis was performed in the Chemical Division, Food Laboratory, JFDA

dark for exactly 5 min. 30ml of distilled water were added. Titration with 0.01N sodium thiosulfate ($Na_2S_2O_3$) was made gradually with constant and vigorous shaking until the yellow color almost disappeared. One ml of (0.1% w/v) starch indicator solution was added. The titration process was continued by shaking the flask vigorously near the end point to liperate all the iodine from the chloroform layer. Thiosulfate was added dropwise until the blue color disappeared. Blank value (must not exceed 0.1ml thiosulfate) was substracted from the sample value. PV, is expressed as mEq O2/kg MT seed oil:

$$PV = \frac{(V_s - V_B) \times N \times 1000}{Wt}$$

Where (V_s and V_B) are titration values for sample and blank, respectively.
N is the normality of thiosulfate, and Wt is the weight of sample.

4-1-3 Oxidative stability index [1]

OSI was determined by using a Rancimat instrument (Model 743; Metrohm Ltd., Switzerland). MT seed oil sample was exposed to accelerated oxidation conditions using the following parameters. Three grams of oil were placed in a reaction vessel, temperature was set at 80C and 100°C in 2 separated channel and the air flow was 20 L/hr. The OSI was measured as the hours (the length of time) for an oil sample to develop measurable rancidity by induction point determination. The OSI measurements were taken in triplicate.

[1] This analysis was performed in the Department of Nutrition and Food Technology, Faculty of Agriculture, University of Jordan

4-2 Instrumental analysis

4-2-1 Fatty acid profile analysis[1]

The fatty acids profile analysis of the MT seed oil was carried out according to the method reported by European Committee (2003). It was determined by conversion of oil to fatty acid methyl esters (FAMEs) followed by gas chromatography as follows:

4-2-1-1 Preparation of fatty acid methyl esters (FAMEs)

About 50 g filtered oil was placed into a screw capped test tube, dissolved in 5 ml hexane (HPLC grade) and mixed by vortex mixer (V1 plus Biosan, Spain) for 60 seconds. A 200µl of 2N potassium hydroxide (85%, GCC laboratory, UK) prepared in anhydrous methanol was added, and mixed for another 30 sec. until solution became clear. Almost immediately after clearance, the solution became turbid due to the separation of glycerol, then 200µl acetic acid was added to prevent the reformation of fatty acids (European Committee, 2003).

4-2-1-2 Gas liquid chromatography analysis of the (FAMEs)

The prepared fatty acid methyl esters (FAMEs) were analyzed on Shimadzu gas chromatograph (Model GC-2010, Shimad zu Inc., Koyoto, Japan) supplied with split injector port (SPL1) and flame ionization detector (FID1). A DB-23 capillary column (Teknokroma, Spain) (60m x 0.25mm internal diameter; film thickness was 0.15µm and the active ingredients were 50% cyanopropyl phenyl and 50% dipheny polysilosan cross linked and chemically bonded) was used. The oven analysis temperature started at 165°C for 8 minute and was raised at a rate of 1°C/minute to 185°C then left at 220°C for 9 minutes.

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[1] This analysis was performed in the Chemical Division, Royal Scientific Society, Jordan.

The injector temperature was 230°C and the detector temperature was 240°C. Flow rate of nitrogen (Carrier gas) was 1.2 ml/minute and split ratio was 1:30. The FAMEs were identified from the chromatogram of a standard solution of FAMEs (Supelco 37 component FAME mixture No. 47885-4, USA). The chromatogram and the peak area were recorded by a digital integrator (Shimodzu C-R8A). Measurements were taken in duplicate. The fatty acid composition was determined as a percent of total fatty acids, using the following equation:

$$\% X \quad \frac{\text{Area for X}}{\text{Total area}} \text{ X } 100\ \%$$

X: is a given fatty acid.

Total area: is sum of areas for all fatty acids.

4-2-2 Unsaponifiable products analysis[1]:

Phytosterols profile and squalene were determined in MT seed oil according to the method reported by European Committee (2003) and Fathi-Achachlouei and Azadmard-Damirchi (2009) with minor modification, as follows:

4-2-2-1 Cold saponification

Accurately, about 200 mg of filtered MT seed oil was weighed into a 15 ml screw capped test tube, 10 ml of 1N KOH (85% GCC Laboratories, UK) dissolved in methanol, 1ml of a squalan solution and 50µg of 5∝ - cholestane as an internal standard (IS) were added to the sample. The mixture was shaken until the diminishing of dispersed oil particles; the sample was then placed in a dark place at room temperature for 22-24 hr (overnight) (Park and Addis, 1986). The Saponified oil was transferred into a 100 ml separatory funnel and 10 ml of distilled water was added. Unsaponifiables were extracted

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[1]This analysis was performed in the Chemical Division, Royal Scientific Society, Jordan.

3 times with 10, 5 and 5ml of diethylether (99% HPLC grade, GCC Laboratories, England).

Pooled diethyl ether extract was washed once with 0.5N KOH (85% GCC laboratories, UK) and 5 times with 5 ml distilled water. The ether was filtered using Whatman No.1 filter paper and dried over anhydrous sodium sulfate (Na_2SO_4) (GCC Laboratories, UK). The filter paper and the anhydrous sodium sulfate were washed twice with 5ml diethyl ether (99% HPLC grade, GCC Laboratories, England), the solvent was concentrated using rotary evaporator (Heidolph, Germany) at 25°C and under vacuum to about 1ml, then dried under ultra pure nitrogen stream after being transferred to a 5 ml vial.

4-2-2-2 Derivatization of the unsaponifiables

The trimethylsilyl derivatives (TMS) of the components of the unsaponifiable were obtained according to Giacometti (2001) as follows: a portion of 500µl of silylation reagent, consisting of a mixture of pyridine (CBH, Nottingham, UK) /hexamethy disilazane (Janssen, Belgium)/trimethylchlorosilane (Fluka, Switzerland), 9: 3:1 (v/v/v) (prepared previously and stored at refrigerator) was added to the unsaponifiable matter, the vial was left for 40 minutes at ambient temperature. The pyridine was evaporated under a stream of extra pure nitrogen, then 4 ml of hexane (GC grade, lab scan, Dublin) was added and shacked vigorously, the solution was transferred into a 5ml screw capped test tube and centrifuged at 4000 rpm for 5 minutes (Hettich EBA-20, Germany). The hexane layer was analyzed immediately by gas chromatography.

4-2-2-3 Gas chromatographic analysis

The TMS derivatives of the unsaponifiables were analyzed on Shimadzu gas chromatograph (model GC-2010, Shimadzu Inc., Koyoto, Japan) supplied with split-split injector port and flame ionization detector, an RTX-65 TG (Restek, USA) CP-SIL 8CB capillary column (30m x 0.25 mm internal diameter; film thickness was 0.25µm; the active

ingredients were 35% diphenyl-65% dimethly polysiloxan) was used. Oven analysis temperature was 265°C and holding time for analysis was 55 minutes. The injector temperature was 280°C, and the detector temperature was 290°C, flow rate of nitrogen (carrier gas) was 1.11ml/minute and split ratio was 1: 30. Squalene and phytosterols profile peaks were identified by comparison with the retention times of reference standard of squalane (Sigma, 98%, GC grade, Japan) and 5∝-Cholestan (Sigma 98%, GC grade, England). The concentration of the analyte was calculated using the internal standard technique (IS) of squalane and 5∝-Cholestane. The chromatogram and the peak area were recorded by a digital integrator (Shimadzu C-R8A). The samples were analyzed in duplicate and the results reported are the means of these.

4-2-3 Total phenols analysis[1]:

The phenol content of MT seed oil was determined following the method of Parry *et al*. (2006) with minor modifications.

4-2-3-1 Extraction and sample preparation:

MT seed oil was extraced with 100% methanol (MeOH) and evaluated for total phenolic contents (TPC) and radical scavenging capacity ($DPPH^\bullet$). Two grams of oil were extracted 3 times by 6ml of MeOH by vortexing for 5 minutes and centrifugation at 6000rpm for 5 minutes. The supernatant was collected and combined then brought to 20ml final volume of extraction solvent (MeOH). Oil extract was kept in the dark under N_2 until analyzed.

4-2-3-2 Total phenolic contents

The TPC of the MT seeds oil was determined using Folin-Ciocalteu (FC, reagent) colorimetric method, based on the reaction of the reagent with the functional hydroxyl

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[1] This analysis was performed in the Chemical Division, Food Laboratory, JFDA.

groups of phenols. Briefly, one ml from the oil extract was placed into a 10ml volumetric flask. Five ml distilled water and 0.25 ml (2N) FC reagent (Sigma-Aldrich, USA) were added and mixed well for 3 minutes. Two ml of Na_2CO_3 (10%) were added and the flask was filled with distilled water up to the mark, mixed and allowed to stand for 60 minute. A calibration curve (Figure7) was prepared using a pure phenolic compound (gallic acid) as a standard, from which serial dilutions were prepared.

The absorbance of the blue color formed was measured at 725 nm in a Perkin Elmer, UV/Vis Spectrophotometer (Perkin Elmer Lambda, 25, 101 NB, USA) against a blank sample.

The TPC was expressed as gallic acid equivalents (GAE) in mg/g of oil. Triplicate measurements were taken.

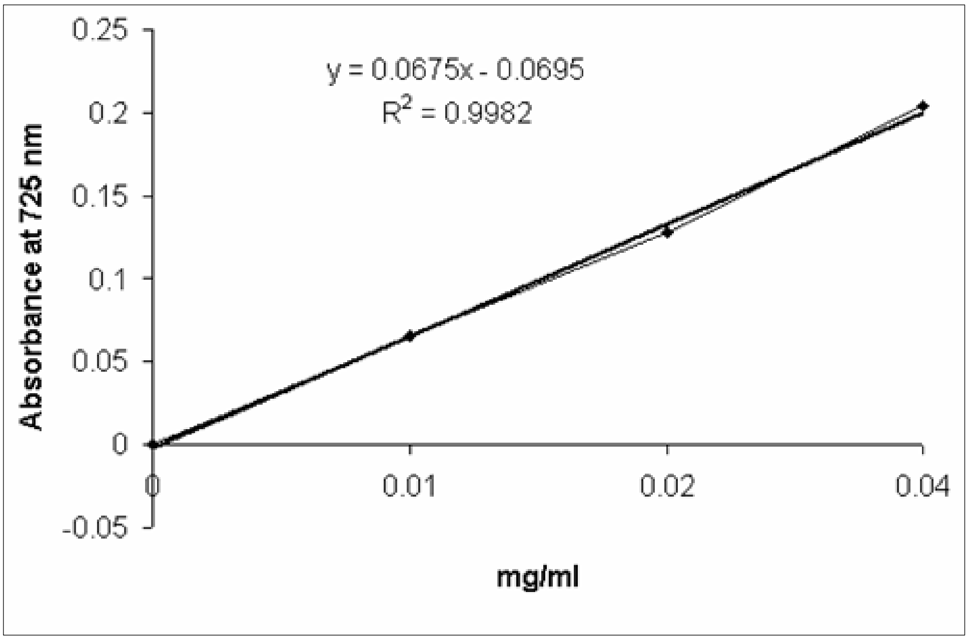


Figure 7: A total phenols absorbance standard curve using gallic acid

4-2-4 Vitamin E (∝- tocopherol)[1].

Vegetable oils are the main dietary sources of vitamin E, which decreases the risk of cardiovascular disease and cancer. ∝-Tocopherol (5, 7, 8-trimethyltocol) is the most active form of vitamin E *in vivo* (Whitney and Rolfes, 2008). The ∝-tocopherol of the MT seed oil sample was analyzed by HPLC according to the method described by Gimeno et al. (2000) as follows:

4-2-4-1 Sample and standard preparation

Stock standard solution of ∝-tocopherol acetate (98%, sigma, USA) was prepared in ethanol, since standard is highly susceptible to oxidation and degradation, stored at -20°C in dark bottles. Working standard solution was prepared from the stock standard solution. To prevent the loss of vitamin E (∝-tocopherol) from the MT seed oil sample, amber-coloured material was used.

One ml of oil sample was diluted in 10 ml of hexane (1: 10). Thereafter 200µl was transferred to a screw-capped tube, where 600µl of methanol and 200µl of the internal standard solution (300 µg/ml of ∝-tocopherol acetate in ethanol) were added. After being vortex-mixed and centrifuged at 3000 rpm for 5 minutes, the sample was filtered through a 0.45 µm pore size filter and an aliquot of the overlay was directly injected into the HPLC.

4-2-4-2 HPLC analysis and quantification

Determination of ∝-tocopherol in oil sample by HPLC was carried out using a Perkin Elmer series 200 (USA) with a PE-1050 pump and 7125 injector model with a final volume loop of 500µl. The detector was a PE-1040M photodiode-array detection (DAD) system. The column was a C-18 (25cm x 4.4mm internal diameter, 5 µm particle size) (Perkin Elmer, USA).

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[1] This analysis was performed in the Chemical Division, Food Laboratory, JFDA.

The injection volume of sample was 50 µl. The mobile phase was methanol: water (96: 4 v/v) (HPLC grade, GCC laboratories, England) and the elution was performed at a flow-rate of 1ml/min. The column was kept at 45°C. Detection was performed at 292 nm. To determine the α-tocopherol in the sample, the working standard solution was analyzed together with the sample and peak-area ratios were used for calculations following the internal standard method. The sample was analyzed in duplicate and the results reported are the mean of these.

4-2-5 Radical (DPPH•) scavenging capacity assay[1]

This method evaluates the radical scavenging capacity of antioxidant compounds by its reaction with the stable radical DPPH•. The assay was carried out in methyl alcohol and the result expressed as IC_{50}, which represent the antioxidant concentration of the extract in mg/ml necessary to scavenge or inhibit the initial DPPH• concentration by 50%.

DPPH• scavenging capacity of the MT seed oil was determined according to the procedure reported by Al-Ismail et al. (2007) and Parry et al. (2008)

4-2-5-1 Extraction and sample preparation

The extraction and sample preparation method is the same mentioned in (4-2-3-1).

4-2-5-2 DPPH• assay

Different aliquots of a methanolic solution of the MT seed oil extract were added to 0.2 ml of DPPH• (ICN Biomedicals Inc. USA) solution (50 mg in 100 ml of methanol) and the volume adjusted (made up) to a final volume of 4 ml with methanol. Six different concentrations (100, 300, 600, 1000, 1200, 1500 µl) were used for assay. The blank sample (0.2 ml of DPPH• solution and the volume adjusted to 4 ml with methanol) was used. After

[1] This analysis was performed in the Department of Nutrition and Food Technology, Faculty of Agriculture, University of Jordan.

a 45 minutes incubation in darkness and at ambient temperature, the absorbance was measured at 515 nm in a spectrophotometer (UV/Vis double beam) (Labomed, INC. USA) against a blank. The inhibition percentage (I%) values were calculated from absorbance of the blank (B) and of the sample (S).

$$\text{Inhibition (\%)} = \frac{B - S}{B} \text{X } 100 \text{ …. [equation of free radical scavenging ratio]}$$

The inhibitory concentration (IC_{50}) value of oil extract was calculated from the dose-response curve by plotting oil extract concentrations against inhibition %. Duplicate measurements were taken.

4-2-6 Total antioxidant capacity [1]

TAC of MT seed oil was measured using the trolox equivalent antioxidant capacity (TEAC) assay. Total antioxidant status (TAS) kit was purchased from Randox Laboratories Ltd. (Crumlin, United Kingdom) and was used to measure TAC of MT seed oil manually on spectrophotometer (UV/Vis double beam, Labomed, Inc., USA).

Test Principle:

ABTS® (2, 2-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 in order to produce the radical cation $ABTS^{\bullet+®}$. This has a relatively stable blue-green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration.

[1] This analysis was performed in the Chemical Division, Food Laboratory, JFDA.

$Hx - Fe^{III} + H_2O_2 \rightarrow X - [Fe^{IV} = O] + H_2O$.
$ABTS^{®} + X - [Fe^{IV} = O) \rightarrow ABTS^{\bullet+®} + HX - Fe^{III}$.
$HX - Fe^{III}$ = methmyoglobin.
$X - [Fe^{IV} = O)$ = Ferryl myoglobin.
$ABTS^{®}$ = 2, 2' – Azino-di-[3-ethylbenzthiazoline sulphonate] it is a registered trademark of Boehringer Mannheim.

Reagents- Working solutions:

Contents	Concentration in the test
R1 - Buffer	
Phosphate buffered saline	80mmol/L, pH 7.4
R2- Chromogen	
Metmyoglobin	6.1 μmol/L
$ABTS^{®}$	610 μ mol/l
R3- Substrate	
H_2O_2	250 μ mol/L
CAL. Standard, Trolox (6-hydroxy-2, 5, 7, 8- tetramethyl chroman -2- carboxylic acid), a vitamin E analogue	2.23 mmol/L

The procedure was as following:

Wavelength	600 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	Against air

Pipette into cuvette:

	Reagent Blank	Standard	Sample
DD H_2O	20 μL	-	-
Standard	-	20 μl	-
Sample (after filtration by using a 0.45μL filter)	-	-	20 μl
Chromogen (R_2)	1ml	1 ml	1 ml

Mix well, incubate to bring to temperature and read initial absorbance A1.

Add**:**	Reagent Blank	Standard	Sample
Substrate R**3**	200 μl	200 μl	200 μl

Mix and start timer simultaneously.
Read absorbance after exactly 3 minutes A_2.
$A_2 - A_1 = \Delta A$ of sample / Standard / Blank.
Calculation;
TAC calculated as TEAC value in (mmol/1)

$$\text{Factor} = \frac{\text{Concentration of standard}}{(\Delta\text{A Blank} - \Delta\text{A Standard})}$$

mmol/l = factor x (ΔA blank - ΔA Standard).

For confirmation of accuracy and reproducibility, a Randox TAS control was used.

5- Preparation of Experimental Diet Mixtures

Two experimental diet mixtures were prepared according to Reeves (1997) that have isonutrient content (isocaloric, isonitrogenous and others). Two levels of dietary oil were used in these diet mixtures: 4% and 8% milk thistle seed oil. Two other diet mixtures (control) were prepared in the same manner but with soybean oil. Four other diet mixtures (2 containing MT seed oil and 2 containing soybean oil as control) were prepared in the same manner but 1% of cholesterol was added to these diets. The recommended fat content in normal diets of adult rats is 4% (Reeves, 1997). Table 4 shows the ingredient composition of the eight experimental diet mixtures. They were formulated by mixing the designated amounts of dietary oils, corn starch, casein, water-soluble vitamins, far-soluble vitamin mix, mineral mix, DL-methionine and choline bitartarate in a stainless steel blender (Kenwood, Hampshire, England). The added amounts of fat (oils) were at the expense of carbohydrates energy in the diet mixtures (by replacing carbohydrate energy with added amounts of dietary oils). Cholesterol was added to diets at the expense of carbohydrate content. No additives, such as antioxidants were added. These diets provided 9.1% and 17.4% of energy from fat respectively. Experimental diet mixtures were freshly prepared once a week, well-packed and placed in dark jars and stored refrigerated at 4ºC until needed during the feeding stage of the experimental animals.

Table (4): Ingredient composition of experimental diets (g/kg)[1-3]

Diet	Without Cholesterol				With Cholesterol			
(Fat %) / Component	Control (4%)	Control (8%)	MTSO (4%)	MTSO (8%)	Control (4%)	Control (8%)	MTSO (4%)	MTSO (8%)
Corn Starch	620.7	580.7	620.7	580.7	610.7	570.7	610.7	570.7
Sucrose	100	100	100	100	100	100	100	100
Cellulose	50	50	50	50	50	50	50	50
Casein	140	140	140	140	140	140	140	140
Soybean oil	36	76	——	——	76	76	——	——
MT seed oil	——	——	36	76	——	——	36	76
Mineral Mix. (1)	35	35	35	35	35	35	35	35
H_2O -soluble vitamin mix (2)	10	10	10	10	10	10	10	10
Fat - soluble vitamin mix (3)	5	5	5	5	5	5	5	5
DL-Methionine	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Choline Bitartarate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Cholesterol	——	——	——	——	10	10	10	10

(1) Mineral mix composed of the following: 357g $CaCO_3$, 250g KH_2 PO_4, 59.11g K_2HPO_4, 74g NaCL, 46g K_2SO_4, 24g MgO, 6.06g $C_6H_5O_7F$. $3H_2O$, 3.79g $ZnSO_4$, 1.45g $Na_2SiO_3.9$ H_2O, 0.92g $MnSO_4$, 0.68g $CuSO_4$, 0.28g CrK $(SO_4)_2$, 0.0815g H_3BO_3, 0.0635g NaF, 0.071g Ni $(NO_3)_2$, 0.0174g Licl, 0.0103g Na_2 SeO_4, 0.010g KIO_3, 0.0069g VCl_3, 0.006g MOO_3. Mixture weight was continued to 1 kg by sucrose (Reeves, 1997).

(2) 1 Kg H_2O – soluble vitamin mix composed of the following: 0.6 g thiamin hydrochloride, 0.6 g riboflavin, 1.6g calcium pantothenate, 3g nicotinic acid, 0.02g biotin, 0.2g folic acid. 0.7g pyridoxine hydrochloride, 0.0025g cyanocobalamin. Mixture weight was contained to 1kg by sucrose (Reeves, 1997).

(3) Fat soluble vitamin mix composed of the following: 0.088g vitamin A (all-trans-retinyl palimtate, 3g vitamin E (α-tocopherol acetate), 0.03 g vitamin K_1 (phylloquinone) 0.001 g vitamin D_2 (Ergocalciferol). All were dissolved in 200 ml soybean oil or MT seed oil (Reeves, 1997).

6- Animal Experimentation

Sprague-Dawley adult male rats (64 rats) with an average weight of 210 g were used. Rats were purchased from the Animal House of the Veterinary Medicine College, Jordan University of Science and Technology in Irbid. In the Animal Unit of the Department of Nutrition and Food Technology at Jordan University, rats were housed in plastic cages with stainless steel wire-mesh bottom. (B. Holden and Crew 2001, North Kent Plsatic cages Ltd, England). They were maintained *ad libitum* on stock diet and tap water for 11 days prior to the start of the experiment for acclimatization. Environmental conditions were under control with a temperature of 22+ 2ºC and 12 hours light-dark cycle. Water was given in glass bottles with rubber stoppers.

At the beginning of the experimental feeding stage, animals were distributed randomly according to their weights into 8 groups (8 rats / group). Each experimental group of animals was assigned to one of the prepared diets for 12 weeks. Both diet and water were provided *ad libitum*. Animal weights and total food intake were measured once a week throughout the experiment. At the end of 12 weeks, rats were starved overnight and were anesthetized by chloroform. Blood samples were collected from the right ventricle of the heart of each rat using a medicinal syringe and transferred to plain tubes, centrifuged at 3200 rpm for 20 minutes (Clement, GS 150 centrifuge, Australia) to obtain serum. Sera were stored in Ependrof plastic tubes, duplicate for each rat, frozen at -20ºC for later analyses of serum TC, HDL-C, LDL-C and TG, TAC and MDA. Livers were excised, cleaned with normal saline (0.9%), and weighed on top loading balance (sartorious, Germany). Weight gain in rats was calculated as follows:

Weight gain = Final weight of rat (g) – Initial weight of rat (g). Food efficiency ratio (FER) was calculated from the weight gain and accumulative food intake at the end of 12 weeks according to the following equation:

FER = Body weight gain (g) /100 g food intake.

7- Biochemical Analysis

7-1 Analysis of lipid profile

The analysis of serum lipids were done in corporation with Al-Khalidi Medical Center Laboratories, Amman, Jordan. COBAS / Integra 400 plus analyzer (Roche Diagnostics GmbH, USA), a clinical chemistry photometric analyzer, was used for the analysis of lipid profile. Prior to analysis, calibration of the analyzer for TC, HDL-C, LDL-C and TG was done according to the manufacturer instructions. The analytical procedures were performed using biochemical kits purchased from Roche, USA. A quality control was run with the samples in each test. Details of the analytical procedures are as follows:

7-1-1 Total cholesterol:

Serum TC of rats was determined by enzymatic-colormetric method, using cholesterol CHOL2 kit (Roche, USA). Principally, cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase (CHE) hydrlolyzes the esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol oxidase (CHOD) according to the following equations:

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest -4-en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Amino phenazone} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{Quinonimine} + 4H_2O$$

A sample volume of 10μL of serum was automatically pipetted from each Ependrof tube, into the tubes of the photometric analyzer, 1 ml of the working reagent which contains (90 mmol/L buffer pH 6.9, 26 mmol/L phenol, 1250 u/L peroxidase, 300 u/L cholesterol esterase, 300u/L cholesterol oxidase and 0.5 mmol/L 4-amino phenazone) was added to the sample, mixed and incubated for 5 minutes at 37ºC. The quantity of red dye quinonimine formed is directly proportional to the concentration of cholesterol. So, the red

color intensity was determined photometrically in the analyzer spetrophotomet by measuring the absorbance (A) of standard and sample against blank reagent a 512 nm.

The concentration of serum TC was calculated automatically as follows:

TC concentration (mg/dL) = A sample x standard concentration (mg/dL) / A standard

7-1-2 High-density lipoprotein – cholesterol

Serum HDL-C of rats was performed using HDLC3 kit (Roche, USA). The test principle was based on the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VDL and chylmicrons are formed which are resistant to polyethylene glycol (PEG) – modified enzymes. The cholesterol concentration of HDL-C is determined enzymatically by CHE and CHOD coupled with PEG to the amino groups (approximately 40%). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by CHE. In the presence of oxygen, cholesterol is oxidized by CHOD to Δ4-cholestenone and H_2O_2. A sample of 9.5 μL of serum was automatically pipetted with 1ml of HDL-C reagent, described above, mixed and incubated at 37ºC for 5 minutes. The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL-C concentration. It is determined by measuring the increase in absorbance (A) at 583 nm. COBAS Integra analyzers automatically calculate the HDL-C concentration of each sample as follows: HDL-C (mg/dL) = A sample x 320

7-1-3 Low density lipoprotein – cholesterol

Serum LDL-C of rats was performed using LDL-C kit (Roche, USA). The principle of this kit is the most recent which is based on the determination of LDL-C after the sample is subjected to immuno- adsorption and centrifugation. This automated method for the direct determination of LDL-C takes advantage of the selective micellary solubilization of LDL-C by anonionic detergent and the interaction of a sugar compound and lipoproteins

(VLDL and chylomicrons). The combination of a sugar compound with detergent by presence of CHE and CHOD coupling reaction enables the selective determination of LDL-C in serum.

LDL-C esters + H_2O $\xrightarrow[\text{CHE}]{\text{detergent}}$ cholesterol + FFA (Selective micellary solubilization)

LDL-C + O_2 $\xrightarrow{\text{CHOD}}$ Δ 4 cholestenone + H_2O_2

2 H_2O_2 + 4-aminoantipyrine + HSDA + H^++ H_2O $\xrightarrow{\text{peroxidase}}$ purple blue pigments + $5H_2O$

9 µL of sample and standard were pipetted and mixed with 1 ml of the reagent solution for each, incubated for 10 minutes at 37ºC. Then the (A) was read at 583 nm, the color intensity of the blue quinonemine dye formed is directly proportional to the LDL-C concentration.

LDL-C was calculated automatically as follows:

LDL-C (mg/dL) = TC – A sample / A standard

7-1-4 Triglycerides

Serum TG of rats was determined by the enzymatic – colorimetric method using TRIGL kit (Roche, USA).

Test principle:

TG + $3H_2O$ $\xrightarrow{\text{Lipoprotein lipase}}$ glycerol + 3 fatty acids

Glycerol + ATP $\xrightarrow[Mg^{+2}]{\text{glycerol kinase}}$ glycerol -3-phosphate +ADP

Glycerol -3-phosphate + O_2 $\xrightarrow{\text{oxidase}}$ dihydroxy acetone – phosphate + H_2O_2

H_2O_2 + 4-aminophenazone + 4- cholorphenol $\xrightarrow{\text{peroxidase}}$ Quinonimine + H_2O + HCl

A sample volume of 30 μL of a serum and 2 ml of working reagent which contains (50mmol/L PIPES buffer pH 6.8, 3.8 mmol/L 4-cholophenol, 150000 U/L LPL, 500 U/L GK, 2500 U/L GPO 440 u/L peroxidase, 0.1 mmol/L 4- aminophenazone and 1.1 mmol/L ATP) were automatically pipette, mixed together and incubated for 4 minutes at 37ºC. The color intensity of the red dye formed is directly proportional to the TG concentration and can be measured photometrically at 512 nm for sample and standard against blank. TG concentration of serum was automatically calculated as follows:

$$\text{TG concentration (mg/dL)} = \frac{\text{A sample}}{\text{A standard}}$$

7-2 Total antioxidant capacity

TAC was measured using TAS kit which was purchased from Randox Laboratories Ltd. (Crumlin, United Kingdom), TAC test was carried on automated chemistry analyzer colorimetric Hitachi 911 (Tokyo, Japan). This analysis was done in corporation with Al-Khalidi Medical Center Laboratories, Amman, Jordan.

Test Principle: ABTS® (2,2-Azino-di[3-ethylbenthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 in order to produce the radical cation $ABTS^{+}$. This has a relatively stable blue-green color, which is measured at 600nm. Antioxidants in the added sample (serum of rats) cause suppression of this color production to a degree which is proportional to their concentration.

$HX\text{-}Fe^{III} + H_2O_2 \rightarrow X\text{-}[Fe^{IV} = o] + H_2O$

$ABTS® + x\text{-} [Fe^{IV} = o] \rightarrow ABTS^{\cdot +} + HX\text{-}Fe^{III}$

$HX\text{-} Fe^{III}$ = metmyoglobin

$X\text{-} [Fe^{IV} = o]$ = Ferrylmyglobin

ABTS ® = 2.2 – Azino- di- [3-ethylbenzthiazoline sulphonate]

ABTS® is a registered trademark of Boehringer Mannheim

Reagent – working solutions:

R1 Buffer (phosphate buffered saline: 80 mmol/L, pH 7.4)

R2 Chromogen (Metmyoglobin: 6.1 µmol/L; ABTS®: 610 µmol/L)

R3 substrate (Hydrogen peroxide (in stabilized form): 250 µmol/L)

CAL. Standard (6-hydroxy-2,5,7,8-tetramethyl chroman 2-Carboxylic acid: 2.23 mmol/L)

For confirmation of accuracy and reproducibility, a Randox TAS control was used.

7-3 Lipid peroxidation

Thiobarbituric acid (TBA) reacts with lipoperoxidation aldehydes, such as malondialdehyde (MDA), as the most common method to assess the end products of lipid peroxidation in biological samples (Jentzch *et al*., 1996; Lapenna *et al*., 2001). In particular, serum TBA test has been used clinically to investigate radical-mediated lipid peroxidation and oxidative stress in disease (Del Rio et al., 2005;Apak *et al*.,2007).

The MDA analysis of the rats serum was carried out according to the method reported by Jentzch et al., (1996) and Labenna et al., (2001) with miner modification. Briefly, 0.5 ml of each serum sample was added to the screw-caped glass tube each containing a reaction mixture (1.0 ml) formed by equal parts of: 15% trichloroacetic acid TCA(7.5 g of TCA to 50 ml of deionized H_2O),0.25 N HCl (25 ml of HCl to 100 ml of deionized H_2O), 0.375% TBA (BDH Laboratory, Supplies, England) (0.375 g of TBA to 100 ml of deionozed H_2O), 2.5 mM BHT (0.0551 g of BHT to 100 ml of ethanol) and a 0.1 ml of 8.1% sodium lauryl sulphate solution (SDS) (dissolving 2.025 of SDS in 25 ml of deionized H_2O), all were mixed by vortex followed by 30 minutes heating at 95ºC; pH value of the analytical reaction mixture was about 1.0. BHT was used to prevent lipid peroxidation during heating. After cooling to room temperature, the chromogen, (TBARS) was extracted with (1.5 ml) n-butanol. To facilitate phase separation, the test tubes were

centrifuged at 3000 rpm for 10 minutes. The upper butanol phase was placed into cuvette and the absorption was read spectrophtometrically (Perkin Elmer lambda 25, 101 N8, USA) at 532 nm against a reaction mixture "blank' lacking serum but subjected to the entire procedure and extracted with n-butanol.To correct for background absorption, absorbance values at 572nm were substracted from those at 532 nm, the latter representing the absorption maximum of the TBARS.

MDA equivalents (TBARS) were calculated using the differences in absorption at the two wavelengths and quantification was made with the application of Beer's Law:

$$C = \frac{A}{\varepsilon \text{ x } i}$$

Where,

C = concentration (mol/L)

A = Absorbance

ε = Molar extinction coefficient for MDA/TBA-complex is 154000 (mol/L).

i = 1 cm light path length of cuvette

Statistical Analysis

Statistical analysis of the measured and calculated data was performed using the statistical analysis system (SAS package,2002) .Standard error of the mean (SEM) were used to describe the study variables. Two and Three way analysis of variance (ANOVA) was used to test mean differences among the eight groups for experimental rats. Tukey test was used to test any differences among means for which ANOVA indicated a significant level ($P < 0.05$).

Results

1. Chemical characterization of cold-pressed MTSO

1-1 Proximate analysis of MT seeds

Table (5) demonstrates proximate analysis of MT seeds.Moisture and protein content were 4.61% and 17.64% respectively. Fat represented the major component in MT seeds (26.9%), while ash and fiber contents were 5.10% and 25.32% respectively.

Table (5): Proximate analysis of MT seed and dry matter basis (1-3)

Component	MT Seeds (%)
Moisture	4.61 + 0.96
Protein	17.64 + 1.12
Fat	26.90 + 1.10
Fiber	25.32 + 1.12
Ash	5.10 + 1.15
NFE	20.43 + 1.10

(1) Results are given as Mean + SD of 2 readings on dry matter basis. (C. V < 0.05).

(2) NFE = Nitrogen -free extract.

(3) The value of NFE are calculated by difference 100 - (protein + Fat + Ash + Fiber).

1-2 Quality indicators of cold- pressed MT seed oil:

The basic quality indicators of cold-pressed MT seeds oil used for the preparation of oil sample used in this study is presented in Table (6).The tested cold - pressed MT seed oil had an acidity of 0.636% as linoleic acid, peroxide value of 0.340 meq O_2/kg of oil.The oxidative stability index (OSI) was determined by induction time. The OSI value of the cold-pressed MT seed oil had an induction time of 55.7 and 12.88 hours at 80°C and 100°C, respectively.

As an example, appendix (1) shows the OSI graph of one run of the cold-pressed MTSO at 100 Ċ.

Table (6): Acidity, peroxide value and oxidative stability index of the oil extracted from cold- Pressed MTseeds [1]

Acidity (Linoleic acid) (%)	**Peroxide value (mEq O_2 /Kg)**	**Oxidative stability index(OSI) (hours)**	
		80 °C	**100 °C**
0.636 ± 0.02	0.340 ± 0.07	55.70 ±0.18	12.88 ± 0.43

(1) Data were expressed as mean ± SD (n= 3) and CV < 5%

1-3 Fatty acid profile in cold-pressed MT seed oil

The fatty acids detected in the cold-pressed MT seed oil were myristic (14:0), palmitic (16:0), palmitoleic (16:1), heptadecanoic (17:0), heptadecenoic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eciosenoic (20:1), behenic (22:0) and lignoceric (24:0) acids (Table 7). The cold-pressed MT seed oil contained a relatively high level of total unsaturated fatty acids that reached about 80.5% from total fatty acids. Linoleic acid (18: 2, n-6) was the most prevalent fatty acid in cold-pressed MT seed oil with 56.79 g per 100g oil. Also, it contained significant concentration of oleic acid (18: 1, n-9) (22.03g/100g oil), and had low concentration of linolenic acid (18: n-3) (0.67g/100g oil). On the other hand, saturated fatty acids (SFA) reached about 19.53% of the total fatty acids. A GLC chromatogram of fatty acids profile is given in appendix (2).

Table (7): Fatty acid composition of oil extracted from cold-pressed MTseeds [(1-2)]

Fatty acids composition		(g/100g)
C14 :0	Myristic acid	0.08 ± 0.001
C16 : 0	Palmitic acid	8.61 ± 0.007
C16 : 1	Palmitoleic acid	0.10 ± 0.001
C17 : 0	Heptadecanoic acid	0.09 ± 0.001
C17 : 1	Heptadecenoic Acid	0.03 ± 0.007
C18 : 0	Stearic acid	5.24 ± 0.001
C18 : 1	Oleic acid	22.03 ± 0.007
C18 : 2	Linoleic acid	56.79 ± 0.03
C18 : 3	Linolenic acid	0.76 ± 0.02
C20 : 0	Arachidic acid	2.88 ± 0.007
C20 : 1	Eicosenoic acid	0.76 ± 0.02
C22 : 0	Behenic acid	2.04 ± 0.007
C24 : 0	Lignoceric acid	0.58 ± 0.007
SFA[(2)]		19.53 ± 0.03
MUFA[(2)]		22.92 ± 0.007
PUFA[(2)]		57.55 ± 0.02

(1) Data were expressed as mean ± SD (n = 2) and CV < 5%

(2) SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

1-4 Phytosterol composition and squalene content in the cold-pressed MT seed oil

Table (8) shows the phytosterol composition and squalene content in the cold-pressed MT seed oil. Fifteen sterols were determined in oil sample, namely:

Cholesterol, 24-methylene-cholesterol, campesterol, campestanol, stigmasterol, delta-7-campesterol, Clerosterol, β-sitosterol, sitostanol, delta-5-avenasterol, delta-5-24-stigmastadienol, delta-7-stigmasterol, delta-7-avenasterol, erythrodiol and uvaol. β-sitosterol was the predominant (1136.3 mg/kg of oil) followed by delta-7-stigmasterol (400,9mg /kg of oil), cholesterol (381.7 mg/ kg of oil), stigmasterol (210:2 mg /kg of oil) and campesterol (167.8 mg /kg of oil). Subsequently, the total phytosterols concentration was (2520 mg/kg of oil). The squalene concentration in the cold-pressed MT seed oil was (9.35 mg/kg of oil) as shown in table (8).

A GLC chromatograms of phytosterols and squaline are given in appendix (3) and (4) respectively.

Table (8): Phytosterols composition and squalene content in the oil extracted from cold - pressed MTseeds [(1-2)]

Sterols	(mg/kg)
Cholesterol	381.7 ± 2.62
Brassicasterol	ND [(2)]
24-methylene-cholesterol	5.80 ± 0.183
Campesterol	167.8 ± 2.12
Campestanol	5.54 ± 0.177
Stigmasterol	210.2 ± 6.40
Delta-7-campesterol	52.42 ± 1.43
Delta-5,23-stigmastadienol	ND
Clerosterol	16.38 ± 0.183
β-sitosterol	1136.3 ± 25.5
Sitostanol	42.10 ± 0.190
Delta-5-avenasterol	14.90 ± 0.00
Delta-5-24-stigmastadienol	38.00 ±0.707
Delta-7-stigmastenol	400.9 ± 16.20
Delta-7-avenasterol	32.50 ± 1.13
Erythrodiol	11.30 ± 0.141
Uvaol	4.03 ± 0.177
Total Phytosterol	2520 ± 0.135
Squalene content (mg/kg)	9.35 ± 0.362

(1)Data were expressed as means ± SD (n = 2) and CV < 5%

(1) ND : not detected

1-5 Antioxidant compounds and capacity of cold-pressed MT seed oil

Total phenolic content (TPC), which plays a dominant role in the antioxidant potential of seed oils, was determined for the cold-pressed MT seed oil extracted by 100% methanol. The value was 1.16 mg gallic acid equivalent/g oil (GAE mg/g) as shown in table (9).In addition, this table shows that the concentration of ∝-tocopherol, one of the important antioxidant compounds in vegetable oils, was 237.4 mg/kg of oil. The HPLC chromatogram of ∝-tocopherol is given in appendix (5).

To further confirm the antioxidant properties of MT seed oil components, the duplicate measurements of cold-pressed MT seed oil were extracted with methanol and the methanol extracts were evaluated for their capacity to directly react with and quench DPPH radicals ($DPPH^{\bullet}$). As shown in table (9), cold-pressed MT seed oil extract exhibited $DPPH^{\bullet}$ scavenging activity under the experiment conditions. After a 45 minutes of antioxidant-$DPPH^{\bullet}$ reactions, MT seed oil extract was able to quench 50% of $DPPH^{\bullet}$ in the reaction mixture at IC_{50} = 3.34 mg/ml..

Also, the TAC estimation was performed to investigate and to further confirm the total potential antioxidant capacity of cold-pressed MT seed oil.

The TAC concentration was 2.29 mmol/L (Table 9).

Table (9). Total phenolic content, vitaminE (as α-tocopherol), radical (DPPH) scavenging capacity and total antioxidant capacity in the oil extracted from cold-pressed MTSO [1]

Type of Test	Values
Total phenolic content [2] (mg GAE/g oil)	1.16 ± 0.008
VitaminE (as α-tocopherol) [3] (mg/kg)	237.4 ± 5.23
Radical (DPPH) scavenging activity (expressed as IC_{50})[3] (mg/ml)	3.34 ± 0.987
Total antioxidant capacity(TAC) [3] (mmol/L)	2.29 ± 0.112

(1) Data were expressed as means ± SD and CV < 5%
(2) Triplicate measurements.
(3) Duplicate measurements.

2- Animal Experimentation

2-1 Body weight, liver weight, accumulative food intake and FER:

Table (10) shows initial and final body weight, body weight gain; liver weights of rats fed two levels of MTSO and control diets with and without cholesterol for twelve weeks. Initial body weights of the group of rats assigned for different diets were essentially similar ($p>0.05$). Rats fed cholesterol-free diets and those fed cholesterol-supplemented diets with two levels of experimental MTSO and control diet exhibited similar ($p>0.05$) final body weights and body weight gain. There were no significant differences ($p>0.05$) between rats within each experimental group of cholesterol-free and cholesterol-supplemented diets regarding liver weights expressed as absolute figures or as g/100g final body weights, except the groups with 8% MTSO and 8% control of cholesterol-supplemented diets were significantly higher ($p<0.05$) than the other groups (Table 10).

Table (11) shows accumulative food intake and FER of rats fed two levels of MTSO and control diets with and without cholesterol for twelve weeks. No significant differences ($p>0.05$) between these variables and experimental diets were observed.

Table (12), (13) and (14) show weekly body weight gain, weekly food intake and weekly FER respectively of rats fed the experimental diets for twelve weeks. During the twelve weeks, there were no significant differences ($p>0.05$) in these variables except the fifth week in weekly FER of rats fed (4%) MTSO of cholesterol free diets, (8%) MTSO and (8%) control of cholesterol- supplemented diets which were significantly different ($p<0.05$). However, the three groups were insignificantly different ($p>0.05$) from those fed the other experimental diets.

Table (10): Initial and final body weights, body weight gain and liver weights of rats fed two levels of MTSO and control diets with and without cholesterol for 12 weeks. (1-2)

Experimental diets \ Body indexes		Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Liver weight (g)	Liver weight /100g final body weight
Cholesterol free diets	MTSO (4%)	212.5 ± 6.7[a]	373.3 ± 12.9[a]	160.7 ± 11.7[a]	9.84 ± 0.68 [b]	2.61 ± 0.10 [c]
	Control (4%)	215.1 ± 7.2[a]	373.5 ± 14.0[a]	158.4 ± 12.6[a]	10.01 ± 0.74 [b]	2.66 ± 0.11 [c]
	MTSO (8%)	211.0 ± 7.9[a]	367.7 ± 15.4[a]	156.6 ± 13.8[a]	9.68 ± 0.81 [b]	2.64 ± 0.12 [c]
	Control (8%)	212.7 ± 7.2[a]	384.1 ± 14.0[a]	171.4 ± 12.7[a]	10.23 ± 0.74 [b]	2.66 ± 0.11 [c]
Cholesterol supplemented diets	MTSO (4%)	214.2 ± 7.2[a]	387.1 ± 14.0[a]	172.9 ± 12.7[a]	12.26 ± 0.74[b]	3.03 ± 0.11 [bc]
	Control (4%)	212.9. ± 7.2[a]	374.2 ± 14.0[a]	161.3 ± 12.7[a]	12.35 ± 0.74 [b]	3.30 ± 0.11 [bc]
	MTSO (8%)	215.0 ± 7.2[a]	408.1 ± 14.0[a]	193.1 ± 12.7[a]	16.20 ± 0.74 [a]	3.98 ± 0.11 [a]
	Control (8%)	214.1 ± 7.2[a]	414.9 ± 14.0[a]	200.9 ± 12.7[a]	17.25 ± 0.74 [a]	4.18 ± 0.11 [a]

(1) Values are given as mean ± SEM for 8 rats.
(2) Values with different superscripts within the same column are significantly different ($P < 0.05$).

Table (11): Accumulative food intake and food efficiency ratio of rats fed two levels of MTSO and control diets with and without cholesterol for 12 weeks[(1-2)]

Experimental diets \ Variables		Accumulative food intake (g)	Food efficiency ratio (FER)
Cholesterol free diets	MTSO (4%)	1571.9 ± 46.6[a]	10.23 ± 0.61[a]
	Control (4%)	1495.2 ± 50.3[a]	10.58 ± 0.65[a]
	MTSO (8%)	1480.4 ± 55.2[a]	10.56 ± 0.72[a]
	Control (8%)	1567.9 ± 50.3[a]	10.90 ± 0.65[a]
Cholesterol supplemented diets	MTSO (4%)	1554.4 ± 50.3[a]	11.10 ± 0.65[a]
	Control (4%)	1549.7 ± 50.3[a]	10.35 ± 0.65[a]
	MTSO (8%)	1613.0 ± 50.3[a]	11.95 ± 0.65[a]
	Control (8%)	1540.8 ± 50.3[a]	12.98 ± 0.65[a]

(1) Values are given as means ± SEM for 8 rats.
(2) Food efficiency ratio is expressed as g body weight gain / 100 g food intake.

Table (12): Weekly body weight gain of rats fed two levels of MTSO and control diets with and without cholesterol for 12 weeks [(1)]

Experimental diets \ Weeks		Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Week 5 (g)	Week 6 (g)	Week 7 (g)	Week 8 (g)	Week 9 (g)	Week 10 (g)	Week 11 (g)	Week 12 (g)
Cholesterol free diets	MTSO (4%)	**33.3± 3.8^{a}**	**18.2±2.9^{a}**	**19.0±3.4^{a}**	**19.1±2.2^{a}**	**13.5±1.5^{a}**	**16.2±1.8^{a}**	**10.8±1.5^{a}**	**12.5±2.1^{a}**	**10.2±1.8^{a}**	**8.0±1.4^{a}**	**8.9±1.4^{a}**	**2.6±1.3^{a}**
	Control (4%)	**29.7 ±4.2^{a}**	**18.4±3.2^{a}**	**16.7±3.6^{a}**	**17.7±2.4^{a}**	**13.0±1.7^{a}**	**13.7±1.9^{a}**	**9.4±1.6^{a}**	**16.5±2.3^{a}**	**9.40±1.7^{a}**	**6.3±1.3^{a}**	**8.7±1.4^{a}**	**2.0±1.2^{a}**
	MTSO (8%)	**30.7 ±4.6^{a}**	**20.6±3.5^{a}**	**16.7±4.0^{a}**	**20.2±2.6^{a}**	**14.4±1.8^{a}**	**15.5±2.1^{a}**	**6.8±1.7^{a}**	**11.0±2.5^{a}**	**9.23±1.6^{a}**	**11.9±1.5^{a}**	**8.9±1.4^{a}**	**1.3±1.1^{a}**
	Control (8%)	**29.7 ±4.2^{a}**	**13.8±3.2^{a}**	**26.7±3.7^{a}**	**17.1±2.4^{a}**	**14.0±1.7^{a}**	**16.7±1.9^{a}**	**9.8±1.6^{a}**	**18.5±2.2^{a}**	**11.2±1.9^{a}**	**7.3±1.6^{a}**	**11.2±1.6^{a}**	**1.9±1.2^{a}**
Cholesterol supplemented diets	MTSO (4%)	**32.4 ±4.2^{a}**	**22.0±3.2^{a}**	**20.7±3.7^{a}**	**18.0±2.4^{a}**	**13.0±1.7^{a}**	**18.1±1.9^{a}**	**7.8±1.6^{a}**	**17.08±2.2^{a}**	**12.6±1.9^{a}**	**8.2±1.6^{a}**	**8.2±1.4^{a}**	**2.9±1.2^{a}**
	Control (4%)	**28.2 ±4.2^{a}**	**19.7±3.2^{a}**	**18.2±3.7^{a}**	**18.7±2.4^{a}**	**12.8±1.7^{a}**	**16.5±1.9^{a}**	**12.7±1.6^{a}**	**16.5±2.2^{a}**	**11.6±1.9^{a}**	**8.9±1.6^{a}**	**6.5±1.6^{a}**	**3.0±1.2^{a}**
	MTSO (8%)	**27.9 ±4.2^{a}**	**25.5±3.2^{a}**	**21.6±3.7^{a}**	**19.8±2.4^{a}**	**18.4±1.7^{a}**	**21.4±1.9^{a}**	**12.6±1.6^{a}**	**17.6±2.2^{a}**	**15.7±1.9^{a}**	**12.7±1.6^{a}**	**12.0±1.4^{a}**	**2.2±1.3^{a}**
	Control (8%)	**29.6 ±4.2^{a}**	**25.4±3.2^{a}**	**20.1±3.7^{a}**	**19.3±2.4^{a}**	**19.7±1.7^{a}**	**21.3±1.9^{a}**	**13.1±1.6^{a}**	**20.2±2.2^{a}**	**16.4±2.0^{a}**	**10.7±1.6^{a}**	**12.6±1.4^{a}**	**7.7±1.3^{a}**

(1) Values are given in means ± SEM for 8 rats.

Table (13): Weekly food intake of rats feed two levels of MTSO and control diets with and without cholesterol for 12 weeks [(1)]

Experemintal diets \ Weeks		Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Week 5 (g)	Week 6 (g)	Week 7 (g)	Week 8 (g)	Week 9 (g)	Week 10 (g)	Week 11 (g)	Week 12 (g)
Cholesterol free diets	MTSO (4%)	135.4 ± 4.8[a]	138.4 ± 5.3[a]	114.0 ± 6.1[a]	126.7 ± 6.3[a]	130 ± 6.8[a]	125.5 ± 6.7[a]	136.1 ± 6.5[a]	136.5 ± 7.0[a]	132.6 ± 5.9[a]	140 ± 6.4[a]	126.5 ± 5.1[a]	129.8 ± 7.0[a]
	Control (4%)	134.4 ± 5.3[a]	135.6 ± 5.7[a]	120.6 ± 6.6[a]	109.8 ± 6.8[a]	113.1 ± 7.39	123.7 ± 7.2[a]	118.4 ± 7.0[a]	129.9 ± 7.6[a]	125.3 ± 6.3[a]	130.9 ± 6.9[a]	124.9 ± 5.6[a]	128.3 ± 7.0[a]
	MTSO (8%)	130.0 ± 5.7[a]	130.9 ± 6.3[a]	113.5 ± 7.2[a]	109.8 ± 7.5[a]	111.7 ± 8.0[a]	120.4 ± 7.9[a]	123.1 ± 7.7[a]	122.9 ± 8.3[a]	117.6 ± 7.0[a]	137.6 ± 7.6[a]	125.8 ± 6.0[a]	136.6 ± 8.3[a]
	Control (8%)	138.8 ± 5.3[a]	133.6 ± 5.7[a]	121.0 ± 6.6[a]	110.5 ± 6.8[a]	119.5 ± 7.3[a]	134.8 ± 7.3[a]	129.5 ± 7.0[a]	147.2 ± 7.6[a]	128.9 ± 6.3[a]	133.5 ± 6.9[a]	138.2 ± 5.6[a]	132.0 ± 7.6[a]
Cholesterol free diets	MTSO (4%)	137.3 ± 5.3[a]	140.6 ± 5.7[a]	117.1 ± 6.6[a]	122.1 ± 6.8[a]	116.9 ± 7.3[a]	135.5 ± 7.3[a]	124.5 ± 7.0[a]	133.2 ± 7.6[a]	124.7 ± 6.3[a]	138.5 ± 6.9[a]	138.3 ± 5.5[a]	125.4 ± 7.6[a]
	Control (4%)	133.3 ± 5.2[a]	126.5 ± 5.7[a]	122.3 ± 6.6[a]	114.1 ± 6.8[a]	110.6 ± 7.3[a]	143.8 ± 7.3[a]	140.7 ± 7.0[a]	133.6 ± 7.6[a]	123.2 ± 6.3[a]	143.4 ± 6.9[a]	130.8 ± 5.5[a]	126.4 ± 7.6[a]
	MTSO (8%)	132.0 ± 5.2[a]	136.7 ± 5.7[a]	115.4 ± 6.6[a]	114.6 ± 6.8[a]	115.5 ± 7.3[a]	149.3 ± 7.3[a]	144.4 ± 7.0[a]	144.4 ± 7.6[a]	142.2 ± 6.3[a]	143.3 ± 6.9[a]	132.5 ± 5.5[a]	142.2 ± 7.6[a]
	Control (8%)	123.0 ± 5.2[a]	127.0 ± 5.7[a]	112.0 ± 6.6[a]	112.1 ± 6.8[a]	119.6 ± 7.3[a]	131.2 ± 7.3[a]	126.2 ± 7.0[a]	134.4 ± 7.6[a]	132.6 ± 6.3[a]	151.9 ± 6.9[a]	140.0 ± 5.5[a]	130.0 ±7.06[a]

(1) Values are given in means ± SEM for 8 rats

Table (14): Weekly food FER of rats feed two levels of MTSO and control diets with and without cholesterol for 12 weeks (1-2)

Experemintal diets	Weeks	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Week 5 (g)	Week 6 (g)	Week 7 (g)	Week 8 (g)	Week 9 (g)	Week 10 (g)	Week 11 (g)	Week 12 (g)
Cholesterol free diets	MTSO (4%)	24.2 ± 2.4 ^{a}	13.1 ± 2.0^{a}	16.5 ± 2.1^{a}	16.2 ± 1.6^{a}	10.5 ± 1.1^{b}	13.2 ± 1.3^{a}	7.74 ± 1.0^{a}	9.35 ± 1.3^{a}	8.42 ± 1.1^{a}	5.23 ± 1.0^{a}	6.80 ± 1.1^{a}	1.87 ± 1.0^{a}
	Control (4%)	22.1 ± 2.5^{a}	13.3 ± 2.1^{a}	13.7 ± 2.3^{a}	16.1 ± 1.7^{a}	11.6 ± 1.2^{ab}	11.1 ± 1.5^{a}	7.83 ± 1.1^{a}	12.6 ± 1.5^{a}	7.60 ± 1.5^{a}	4.70 ± 1.1^{a}	6.98 ± 1.1^{a}	1.46 ± 1.1^{a}
	MTSO (8%)	23.5 ± 2.8^{a}	15.7 ± 2.3^{a}	14.8 ± 2.5^{a}	18.4 ± 1.9^{a}	12.8 ± 1.3^{ab}	12.8 ± 1.6^{a}	5.44 ± 1.2^{a}	9.2 ± 1.6^{a}	7.00 ± 1.3^{a}	9.70 ± 1.2^{a}	7.54 ± 1.2^{a}	1.04 ± 1.2^{a}
	Control (8%)	21.3 ± 2.6^{a}	10.2 ± 2.1^{a}	21.2 ± 2.3^{a}	15.5 ± 1.7^{a}	11.5 ± 1.2^{ab}	12.7 ± 1.4^{a}	7.73 ± 1.1^{a}	12.6 ± 1.5^{a}	8.90 ± 1.2^{a}	5.54 ± 1.2^{a}	8.33 ± 1.1^{a}	1.38 ± 1.1^{a}
Cholesterol supplemented diets	MTSO (4%)	23.3 ± 2.5^{a}	15.6 ± 2.1^{a}	17.2 ± 2.3^{a}	14.8 ± 1.8^{a}	11.4 ± 1.5^{ab}	13.3 ± 1.5^{a}	6.71 ± 1.1^{a}	12.6 ± 1.5^{a}	10.2 ± 1.2^{a}	6.16 ± 1.1^{a}	5.96 ± 1.1^{a}	2.18 ± 1.1^{a}
	Control (4%)	20.8 ± 2.5^{a}	15.2 ± 2.1^{a}	14.3 ± 2.3^{a}	16.3 ± 1.8^{a}	11.9 ± 1.2^{ab}	11.9 ± 1.5^{a}	9.35 ± 1.1^{a}	12.8 ± 1.5^{a}	9.25 ± 1.2^{a}	6.36 ± 1.1^{a}	5.10 ± 1.1^{a}	2.73 ± 1.1^{a}
	MTSO (8%)	20.6 ± 2.5^{a}	18.8 ± 2.1^{a}	18.4 ± 2.3^{a}	17.6 ± 1.8^{a}	16.0 ± 1.2^{a}	14.5 ± 1.5^{a}	8.93 ± 1.1^{a}	12.01 ± 1.5^{a}	11.2 ± 1.2^{a}	7.20 ± 1.1^{a}	5.78 ± 1.1^{a}	5.66 ± 1.1^{a}
	Control (8%)	23.6 ± 2.5^{a}	19.9 ± 2.1^{a}	18.1 ± 2.3^{a}	17.4 ± 1.8^{a}	16.3 ± 1.2^{a}	16.4 ± 1.5^{a}	10.6 ± 1.1^{a}	15.1 ± 1.5^{a}	12.6 ± 1.2^{a}	8.65 ± 1.1^{a}	8.63 ± 1.1^{a}	1.78 ± 1.1^{a}

(1) Values are given in means ± SEM for 8 rats

(2) Values with different superscript within the same column are significantly different ($P < 0.05$)

Irrespective of cholesterol (table 10-a), non of the animal groups (n=16) did the experimental diets affect significantly the final body weights, body weight gain, accumulative food itake, FER, liver weight and liver weight /100g final body weight(p>0.05). When considering the effect of cholesterol and the % of both oil used (irrespective of oil type), there were significant differences (p<0.05) between (4%oil) cholesterol-free diet and (8%oil) cholesterol-supplemented diet regarding body weight gain and FER. Also, liver weight and liver weight/100g of final body weight were highly significant between groups fed cholesterol supplemented diets and groups fed cholesterol –free diets. Within the cholesterol –supplemented diets there was a significant difference between groups fed 4% and 8% oil (table 10 –b).

When studying the effect of cholesterol and type of oil (MTSO and control oil diet) irrespective of oil % (Table 10-c), there were highly significant differences (p<0.05) between the groups fed cholesterol –free diets and the groups fed cholesterol-supplemented diets regarding liver weight and liver weights /100 g of final body weights. Results were in agreement with those obtained when type of oil was not considered, for the same variables (Table 10-b). Subsequently when taking into consideration the effect of oil type and the % of the oil (irrespective of cholesterol) there were no significant differences (p>0.05) between all experimental diets (Table 10-a).

Table (10, a) : Body weight gain, accumulative food intake, FER and liver weight of rats fed two levels of MTSO and Control oil (irrespective of cholesterol added to the diet)[(1-2)]

Experimental diet		**Body weight gain (g)**	**Accumulative food intake (g)**	**FER**	**Liver weight (g)**	**Liver weight/100g final body weight**
MTSO	**4%**	166.3 ± 8.88 [a]	1563.8±34.2 [a]	10.6±0.47 [a]	10.9± 0.87 [a]	2.8±0.17 [a]
Control oil	**4%**	159.8±9.24[a]	1522.5±35.6[a]	10.4±0.48 [a]	11.2±0.90 [a]	3.0±0.18 [a]
MTSO	**8%**	176.5±9.66[a]	1552.7±37.1[a]	11.3±0.51[a]	13.2±0.94[a]	3.3±0.19[a]
Control oil	**8%**	181.1±9.24 [a]	1554.4±35.6[a]	12.0±0.48[a]	13.6±0.90[a]	3.4±018 [a]

(1) Values are given as means ± SEM for 16 rats.

(2) Food efficiency ratio is expressed as g body weight gain / 100 g food intake.

Table (10, b): Body weight gain, accumulative food intake, FER and liver weight of rats fed two levels of MTSO and control oil with and without cholesterol (irrespective of oil type)[(1-2)]

Experimental diet		Body weight gain (g)	Accumulative food intake (g)	FER	Liver weight (g)	Liver weight/100g final body weight
Cholesterol-free diets	**4 %**	159.6 ± 8.62 [b]	1533.6± 34.3[a]	10.4±0.44 [b]	9.9±.50[c]	2.6±0.07[c]
	8%	164.0±9.38[ab]	1524.1±37.3[a]	10.7±0.48 [ab]	9.9±0.55[c]	2.6±0.08 [c]
Cholesterol - supplemented diets	**4%**	167.1±8.95[ab]	1522.0±35.6[a]	10.7±0.46 [ab]	12.3±0.52[b]	3.2±0.08 [b]
	8%	196.9±8.95 [a]	1576.9±35.6[a]	12.5±0.46[a]	16.7±0.52[a]	4.1±0.08 [a]

(1) Values are given as means ± SEM for 16 rats.

(2) Food efficiency ratio is expressed as g body weight gain / 100 g food intake

Table (10, c): Body weight gain, accumulative food intake, FER and liver weight of rats fed MTSO and Control oil with and without cholesterol (irrespective of oil level) (1-2)

Experimental diet		Body weight gain (g)	Accumulative food intake (g)	FER	Liver weight (g)	Liver weight/100g final body weight
Cholesterol- free diets	MTSO	158.7 ± 9.07 [a]	1526.1± 36.1[a]	10.4±0.47 [a]	9.7± 0.53[b]	2.6±0.08[b]
	Control oil	165.0±8.95[a]	1531.6±35.6[a]	10.7±0.46 [a]	10.1±0.52[b]	2.7±0.08 [b]
Cholesterol-supplemented diets	MTSO	183.0±8.95[a]	1583.7±35.6[a]	11.5±0.46 [a]	14.2±0.52[a]	3.5±0.08 [a]
	Control oil	181.0±8.95 [a]	1545.3±35.6[a]	11.7±0.46[a]	14.8±0.52[a]	3.7±0.08 [a]

(1) Values are given as means ± SEM for 16 rats.

(2) Food efficiency ratio is expressed as g body weight gain / 100 g food intake

2-2 **Levels of blood lipids and lipoprotein**

Table (15) shows the concentration of blood lipids and lipoprotein of rats fed two levels of MTSO and control diets with and without cholesterol for twelve weeks. With respect to serum levels of TC expressed as (mg/dL), there were significant differences ($p<0.05$) between rats fed cholesterol-supplemented diets with 8% MTSO and 8% control oil and cholesterol-free diets with 8% control oil. Also, cholesterol-supplemented diet with 8% control oil was significantly higher ($p<0.05$) than those with 4% MTSO added to cholesterol-free diets. Regarding HDL-C levels, no significant differences ($p>0.05$) were detected between rats fed two levels of MTSO and control oil with cholesterol –supplemented diets (Table 15). However, rats fed diets containing 4% and 8% MTSO exhibited significantly higher ($p<0.05$) levels of HDL-C as compared with rats fed 8% control oil within cholesterol –free diet groups. Higher significant ($p<0.05$) differences in HDL-C concentration were also observed between rats fed cholesterol –free diets and rats fed cholesterol diets.

On the other hand, serum levels of LDL-C of rats fed the cholesterol –free diets were significantly lower ($p<0.05$) than those fed the cholesterol-supplemented diets (Table 15). Regarding TG (Table 15), rats fed the cholesterol –free diet containing 4%MTSO exhibited significantly lower ($p<0.05$) levels in comparison with those fed 4 % control oil diet. Also, the latter diet group was significantly higher ($p>0.05$) than other rat groups fed cholesterol-supplemented diet and cholesterol-free diet.

Table (15): Concentration of blood lipids and lipoproteins of rats fed two levels of MTSO and control diets with and without cholesterol for 12 weeks (1-2).

Experimental diets		Blood lipids and lipoproteins (mg/dL)			
		Total cholesterol	High density lipoprotein -cholesterol	Low density lipoprotein -cholesterol	Triglycerides
Cholesterol free diets	MTSO (4%)	66.5 ± 7.0^{bc}	75.3 ± 3.9^{a}	16.6 ± 5.3^{c}	54.6 ± 8.3^{b}
	Control (4%)	86.8 ± 7.6^{abc}	72.4 ± 4.3^{ab}	18.2 ± 5.7^{c}	106.0 ± 9.0^{a}
	MTSO (8%)	89.0 ± 8.3^{abc}	76.4 ± 4.6^{a}	18.2 ± 6.3^{c}	75.6 ± 9.8^{ab}
	Control (8%)	59.8 ± 7.6^{c}	60.1 ± 4.2^{b}	15.0 ± 5.7^{c}	60.7 ± 9.0^{b}
Cholesterol supplemented diets	MTSO (4%)	79.0 ± 7.6^{abc}	48.3 ± 4.2^{c}	49.4 ± 5.7^{ab}	52.8 ± 9.0^{b}
	Control (4%)	71.2 ± 7.6^{abc}	43.0 ± 4.2^{c}	40.5 ± 5.7^{b}	58.8 ± 9.0^{b}
	MTSO (8%)	97.7 ± 7.6^{ab}	52.5 ± 4.2^{c}	66.7 ± 5.7^{a}	44.0 ± 9.0^{b}
	Control (8%)	104.8 ± 7.6^{a}	42.2 ± 4.2^{c}	71.6 ± 5.7^{a}	50.2 ± 9.0^{b}

(1) Values are given as mean ± SEM for 8 rats

(2) Values with different superscript within the same column are significantly different ($P < 0.05$).

Table (16) shows indices of blood lipids and lipoprotein of rats fed two levels of MTSO and control diets with and without cholesterol. High significant differences ($p<0.05$) in HDL-C/LDL ratio between rats fed cholesterol-free diet and those fed cholesterol – supplemented diets were indicated. Rats fed the diet containing 8% MTSO and 8% control diet with cholesterol had significantly higher ($p<0.05$) TC/TG ratio than all cholesterol-free diets (Table 16).

Atherogenic index (AI) of the eight experimental diet groups was calculated as (TC – HDL-C)/HDL-C (Martin –Carron *et al.*, 1999). It was observed that there were no significant differences ($p>0.05$) among rats fed cholesterol-free diets (Table 16). However, rats fed the cholesterol-supplemented diet with 8% MTSO and 8% control had the highest AI among all cholesterol- containing and cholesterol- free diet groups.

Table (16): Indices of blood lipids and lipoproteins of rats fed two levels of MTSO and control diets with and without cholesterol for 12 weeks [(1-3)].

Experimental diets		HDL-C/LDL-C ratio	TC/TG Ratio[(4)]	Atherogenic Index (AI)
Cholesterol -free diets	MTSO (4%)	4.64 ± 0.20[a]	1.30 ± 0.25[bc]	0.15 ± 0.13[c]
	Control (4%)	4.07 ± 0.23[a]	0.89 ± 0.27[c]	0.19 ± 0.14[c]
	MTSO (8%)	4.47 ± 0.24[a]	1.15 ±0.29[bc]	0.15 ± 0.16[c]
	Control (8%)	3.73 ± 0.22[a]	1.11 ± 0.27[c]	0.07 ± 0.14[c]
Cholesterol-supplemented diets	MTSO (4%)	1.17 ± 0.22[b]	1.47 ± 0.27[abc]	0.67 ± 0.14[bc]
	Control (4%)	1.17 ± 0.22[b]	1.27 ± 0.27[abc]	0.66 ± 0.14[bc]
	MTSO (8%)	0.85 ± 0.22[b]	2.48 ± 0.27[a]	1.00 ± 0.14[a]
	Control (8%)	0.62 ± 0.22[b]	2.40 ± 0.27[a]	1.51 ± 0.14[a]

(1) Values are given as mean ± SEM for 8 rats

(2) Values with different superscript within the same column are significantly different ($P < 0.05$).

(3) Atherogenic index = (TC – HDL-C) X HDL-C $^{-1}$ (Martin –Carron et al.,1999)

(4) An indicator of atherogenicity.

Irrespective of cholesterol (Table 17, a), none of the animal groups did the experimental diets affect significantly the TC, HDL-C, LDL-C, HDL-C/LDL-C ratio, TC/TG ratio, AI and TAC ($p>0.05$). However, there were significantly higher accumulations of MDA in the rats fed 8% control oil diet as compared with the other groups. Also, TG level was significantly higher in rats on control diet with 4% of oil compared with the other groups.

Table (17, b) shows the effect of cholesterol and the % of oil (irrespective of oil type). Blood total cholesterol was significantly higher in rats fed cholesterol -supplemented diet with 8 % oil. HDL-C was significantly higher in groups fed cholesterol free diets compared to those fed cholesterol –supplemented diets. On the other hand, LDL-C was significantly higher in group's fed cholesterol-supplemented diets. These results are in accordance with HDL-C/LDL-C ratio which was significantly higher in cholesterol –free diets .TG was significantly higher in groups fed 4 % oil of cholesterol-free diet when compared to other groups. TC/TG ratio was significantly higher in rat's fed 8% cholesterol – supplemented. A high and significant value was found for AI in group fed 8% cholesterol –supplemented diet. MDA was significantly higher in groups fed cholesterol-supplemented diets when compared to the 4% cholesterol free diets.

When considering the effect of cholesterol and type of oil (MTSO and control oil diet), irrespective of oil level (Table 17, c), there were insignificant differences ($p>0.05$) between all experimental diets regarding TC. On the other hand, serum levels of HDL-C of rats fed the cholesterol –free diets were significantly higher ($p<0.05$) than those fed the cholesterol-supplemented diets. Among rats fed cholesterol-free diets, HDL-C level was highly significant ($p<0.05$) in the group fed MTSO than the control oil group. Also, when serum levels of LDL-C were investigated, rats fed the cholesterol –free diet exhibited significantly lower ($p<0.05$) levels than those fed cholesterol –supplemented diets .With

respect to TG, rats fed cholesterol- free diet with control oil had significantly higher values than rats fed cholesterol-supplemented diets with MTSO and control oil. MTSO diet (without cholesterol supplemented) was significantly higher ($p<0.05$) than all experimental diets regarding HDL-C/LDL-C ratio. TC/TG ratio was significantly lower in the group fed control oil of cholesterol-free diet when compared to the group fed MTSO and control oil of cholesterol – supplemented diets. When taking into consideration the AI, there were high significant differences ($p<0.05$) between groups fed cholesterol-supplemented diets and groups fed cholesterol –free diet. Regarding TAC, there were high significant differences between MTSO and control oil within the cholesterol–free diet. The difference was also significantly higher between MTSO-cholesterol free group and MTSO-cholesterol supplemented group.

High significant differences ($p<0.05$) in MDA concentration between rats fed MTSO and control oil of cholesterol –free diets were indicated. Also, the same effect was observed between MTSO group and control oil group of cholesterol- supplemented diet .Subsequently when taking into consideration the effect of oil type, MTSO group of cholesterol-free diet was significantly lower ($p<0.05$) than those fed the cholesterol-supplemented diets, particularly control oil group which had a high concentration of MDA.

Table (17. a): Concentration and indices of blood lipids and lipoproteins, TAC and MDA of rats fed two levels of MTSO and Control oil (irrespective of cholesterol) (1-3)

Experimental diet		TC	HDL-C	LDL-C	TG	HDL-C/LDL-C ratio	TC/TG ratio	AI	TAC (mmol/L)	MDA (nmol/ml)
MTSO	4%	72.3±6.1[a]	62.8±4.5[a]	30.8±7.0[a]	53.7±7.2[b]	3.0±0.5[a]	1.4±0.2[a]	0.4±0.2[a]	1.3±0.03[a]	7.4±0.6[b]
Control oil	4%	79.0±6.4[a]	57.7±4.6[a]	27.8±7.3 [a]	82.4±7.4[a]	2.6±0.5[a]	1.1±0.2[a]	0.4±0.2[a]	1.2±0.03[a]	9.6±0.6[b]
MTSO	8%	93.7±6.7 [a]	63.4±4.8 [a]	44.6±7.6[a]	58.3±7.8[b]	2.5±0.5[a]	1.9±0.2 [a]	0.6±0.1[a]	1.3±0.03[a]	7.0±0.7[b]
Control oil	8%	82.3±6.4 [a]	48.9±4.6 [a]	43.3±7.3[a]	55.4±7.4[b]	2.1±0.5[a]	1.8±0.2[a]	0.8±0.1[a]	1.2±0.03[a]	12.2±0.6[a]

(1) Values are given as means ± SEM for 16 rats.

(2) TAC: total antioxidant capacity.

(3) MDA : malondialdehyde.

Table (17.b): Concentration and indices of blood lipids and lipoproteins, TAC and MDA of rats fed two levels of MTSO and control oil with and without cholesterol (Irrespective of oil type) [(1-3)]

Experimental diet		TC	HDL-C	LDL-C	TG	HDL-C/LDL-C ratio	TC/TG ratio	AI	TAC (mmol/L)	MDA (nmol/ml)
Cholesterol free diets	**4 %**	76.7±2.1 ^{b}	73.9±2.90 ^{a}	17.4±3.93 ^{c}	80.3±6.14 ^{a}	4.35±0.15 ^{a}	1.0±0.18 ^{b}	0.2±0.1 ^{c}	1.3±0.03 ^{a}	7.3±0.58 ^{b}
	8 %	74.4±2.2 ^{b}	66.0±3.16 ^{a}	16.6±4.28 ^{c}	61.6 ±6.69 ^{b}	4.10±0.16 ^{a}	1.2±0.20 ^{b}	0.1±0.1 ^{c}	1.2±0.03 ^{a}	9.4±.63 ^{ab}
Cholesterol supplemented diets	**4 %**	75.0±2.6 ^{b}	45.6±3.01 ^{b}	42.4±4.08 ^{b}	55.8±6.37 ^{b}	1.17±0.16 ^{b}	1.4±0.19 ^{b}	0.7±0.1 ^{b}	1.2±0.03 ^{a}	9.8±0.60 ^{a}
	8 %	101.2±2.5 ^{a}	47.4±3.01 ^{b}	69.1±4.08 ^{a}	47.0±6.37 ^{b}	0.74±0.16 ^{b}	2.5±0.19 ^{a}	1.3±0.1 ^{a}	1.3±0.03 ^{a}	9.7±0.60 ^{a}

(1) Values are given as means ± SEM for 16 rats.

(2) TAC: total antioxidant capacity.

(3) MDA: malondialdehyde.

Table (17.c): Concentration and indices of blood lipids and lipoproteins, TAC and MDA of rats fed MTSO and Control oil with and without cholesterol (irrespective of oil level) (1-3)

Experimental diet		TC	HDL-C	LDL-C	TG	HDL-C/LDL-C ratio	TC/TG ratio	AI	TAC (mmol/L)	MDA (nmol/ml)
Cholesterol-free diets	MTSO	77.8±5.5^{a}	75.9±3.1^{a}	17.4±4.2^{b}	65.0±6.5^{ab}	4.6±0.16^{a}	1.1±0.2^{b}	0.1±0.1^{b}	1.4±0.03^{a}	6.7±0.6^{c}
	Control oil	73.3±5.4^{a}	64.0±3.0^{b}	16.6±4.1^{b}	83.3±6.4^{a}	3.8±0.15^{b}	1.0±0.2^{b}	0.1±0.1^{b}	1.2±0.03^{b}	9.1±0.6^{b}
Cholesterol-supplemented diets	MTSO	88.3±5.4^{a}	50.4±3.0^{c}	57.0±4.1^{a}	48.4±6.4^{b}	1.0±0.15^{c}	1.9±0.2^{a}	0.8±0.1^{a}	1.2±0.03^{b}	7.8±0.60^{bc}
	Control oil	88.0±5.4^{a}	42.6±3.0^{c}	54.6±4.1^{a}	54.5±6.4^{b}	0.9±0.15^{c}	1.8±0.2^{a}	1.1±0.1^{a}	1.2±0.03^{b}	11.7±0.6^{a}

(1) Values are given as means ± SEM for 16 rats.

(2) TAC: total antioxidant capacity.

(3) MDA: malondialdehyde.

2-3 Total antioxidant capacity:

Table (18) shows total antioxidant capacity (TAC) of rats fed two levels of MTSO and control diets with and without cholesterol for twelve weeks. Rats fed the diet containing 4% MTSO without cholesterol had significantly higher ($p<0.05$) TAC than those on 8% control oil diet without cholesterol and those on 4 % MTSO diet with cholesterol.

Table (18): Total Antioxidant capacity of rats fed two levels of MTSO and control diets with and without cholesterol for a period of 12 weeks.

Experimental diets		**Total Antioxidant capacity (mmol/L)**
Cholesterol- free diets	MTSO (4%)	1.41 ± 0.041^{a}
	Control (4%)	1.27 ± 0.044^{ab}
	MTSO (8%)	1.32 ± 0.048^{ab}
	Control (8%)	1.15 ± 0.044^{b}
Cholesterol - supplemented diets	MTSO (4%)	1.21 ± 0.044^{b}
	Control (4%)	1.25 ± 0.044^{ab}
	MTSO (8%)	1.23 ± 0.044^{ab}
	Control (8%)	1.26 ± 0.044^{ab}

2-4 Malondialdehyde:

When serum levels of malondialdehyde (MDA) were investigated (Table19), rats fed the cholesterol- free diet containing 8% control oil exhibited significantly higher ($p<0.05$) levels than those fed other cholesterol-free diets. MDA levels were significantly higher ($p<0.05$) in rats fed 8% control oil of the cholesterol-supplemented diet when compared with rats fed 4% control oil, 4% MTSO and 8% MTSO with cholesterol and rats on 4% MTSO, 4% control oil and 8% MTSO of cholesterol –free diets .

Table (19): Malondialdehyde levels of rats feed two levels of MTSO and control diets with and without cholesterol for a period of 12 weeks.

Experemintal diets		**Malondialdehyde (nmol/ml)**
Cholesterol -free diets	MTSO (4%)	6.51 ± 0.78^{c}
	Control (4%)	$7.94 \pm 0.85b^{bc}$
	MTSO (8%)	6.88 ± 0.93^{c}
	Control (8%)	12.10 ± 0.85^{a}
Cholesterol - supplemented diets	MTSO (4%)	8.21 ± 0.85^{bc}
	Control (4%)	9.56 ± 0.88^{b}
	MTSO (8%)	7.27 ± 0.85^{c}
	Control (8%)	12.18 ± 0.85^{a}

DISCUSSION

1. Chemical Characterization of Cold-pressed MTSO

1-1 Proximate analysis of MT seeds

As seen in table (5), fat represented the major component in MT seeds (26.90%). This result is within the range found by Fathi-Achachlonei and Azadmard (2009), which was (25-31%). The proximate analysis of MT seeds also agreed with those reported by Abu-Rajouh (1996) and with those reported by Abu-Jadayil *et al*. (1999) see table (2).

1-2 Quality indicators of cold-pressed MTSO

It is obvious that the highest quality MTSO was obtained by the technique of cold-pressing of seeds. This has been proven by basic chemical analysis of acidity % and peroxide value as quality parameters of cold-pressed MTSO (Table 6). The acidity % and peroxide value of cold-pressed MTSO were 0.636% and 0.340 meq O_2/kg of oil, respectively. These values were lower than the maximum values permitted for cold-pressed and virgin oils prescribed by the Codex Standards for vegetable oils (0.8% acidity and 15 mEq O_2/kg oil as PV) (Joint FAO/WHO, 2005).

The very low acidity % (<1) and peroxide value (<15 mEq O_2/kg oil) in cold-pressed oils indicate that there was no chemical or enzymatic hydrolysis of glycerides and also indicate the freshness of the sample and the good quality of oil (Satil *et al*., 2003; Arena *et al*., 2007). These results are in agreement with data of cold-pressed seed oil reported in the literature. Arena *et al*. (2007) reported that the acidity % and peroxide value of the cold-pressed pistachio nut oil were 0.66% and 5.7mEq/kg, respectively. Also, values of 0.6-0.9% and 1.5-6.5mEq/kg respectively were reported for five varieties of cold-pressed oils (Nyam *et al*., 2009). The lower acidity of these seed oils indicate that they are edible and could have a long shelf life (Arena *et al*., 2007, Nyam *et al*., 2009).

OSI measures the volatile secondary products of the free radical mediated lipid peroxidation and gives an indication of the shelf life. Greater OSI value is associated with a longer shelf life (Lutterodt *et al*. 2010). As seen in table (6), OSI value gave a longer shelf life for cold-pressed MTSO at 80°C, while the OSI of cold-pressed MTSO at 100°C gave the shorter shelf life, reflecting a 4.5-fold difference in their OSI. Thus, is an inverse relation between the temperature used in OSI test and hours of induction period of oxidation.

At 80°C, the value of cold-pressed MTSO (55.7 hr) was higher than that of 47 hr for commercial soy bean oils and lower than that of about 66.8 hr for corn oils. This value was also higher when compared with the same type of oil (MTSO) where it gives a value of 14 hr as reported by Parry, *et al*. (2006). The difference in this value between the same type of oil (cold-pressed MTSO) may be due to different experimental conditions like an air flow of 7 L/hr in Parry study while it was 20 L/hr in this study.

Also, these differences may be due to geographical, climatic differences of areas where the MT seeds had been grown and influence of chemical components in MT seeds that affects the quality of oil.

Lutterodt *et al* (2010) suggested that the level of unsaturated fatty acids present in the oils plays a determining role in their oxidative stability, with higher unsaturation associated with the lower OSI. Also, they suggested that other factors such as the contents of polyphenolics and other naturally-occurring antioxidants in the oil such as ∝-tocopherol contribute to oxidative stability.

1-3 Fatty acid profile in cold-pressed MTSO

Interestingly, the number of fatty acids detected in the present study (13 fatty acids) was more than those detected by two recent studies, Parry *et al*. (2006) (8 fatty acids) and Fathi-Achachlouei and Azadmard-Damirchi (2009) (9 fatty acids) in MTSO.

The primary fatty acid in the cold-pressed MTSO was linoleic acid followed by oleic acid and palmitic acid (Table 7). These results agreed, with slight differences with those reported by Kaezmarek and Myrugasiewiez (1975), Hamed, *et al*. (1983), Khan, *et al*. (1985), Abu-Rajouh (1996), parry *et al*.(2006) as shown in Table (3). These slight differences may be due to geographical and climatic differences of areas where the MT seeds had been grown.

When compared with other seed oils, cold-pressed MTSO contained about 19.5g of SFA/100g total fatty acids, which is much lower than that of 30.8 g/100g total fatty acids in the cold-pressed cardamom seed oil and comparable to that of 17.3, 20.0 g/100g total fatty acids found in the cold-pressed grape seed oil and sunflower seed oil, respectively (Parry *et al*., 2006; Tuberoso *et al*., 2007).

This saturated fat level was higher than that of 7.4-9.7 g/100g total fatty acids in the cold-pressed Parsley, onion, hemp, Mullein, and cranberry seed oils (Parker *et al*., 2003; Parry *et al*., 2006).

MTSO contained significant level of MUFA's (about 22.9g/100g total fatty acids), which is comparable to the cold-pressed hemp, cranberry, blue berry, onion, flax seed, sunflower and black cumin seed oils but was much lower than that of 81 and 82% in the cold-pressed carrot and parsley seed oils (Parker *et al*., 2003; Parry *et al*., 2005; 2006; Lutterodt *et al*., 2010). MTSO had a PUFA content of 57.6g/100g total fatty acids. This PUFA content was comparable to that in the cold-pressed black cumin (58.7g/100g), sunflower (57.2g/100g) and cotton (56.6g/100g) seed oils, but lower than that in the cold-

pressed red raspberry, marion- berry, hemp, boysenberry and mullein seed oils with a PUFA content of 75-89g/100g total fatty acids (Parker *et al*., 2003 Aguilera *et al*., 2004., Parry *et al*., 2005; 2006; Tuberoso *et al*., 2007; Lutterodt *et al*., 2010).

As a result, the fatty acid profile of MTSO in the present study seems to be similar to that of sunflower seed oil and black cumin seed oil.

1-4 Phytosterol composition and squalene content in the cold-pressed MT seed oil

Phytosterols (plant sterols) are minor components of all vegetable oils and form a major proportion of the unsaponfiables (Lagarda *et al*., 2006; Nyam *et al*., 2009). Phytosterols are important from a health point of view because they contribute to the lowering of serum cholesterol levels in humans (Fathi-Achachlouei and Azadmard-Damirchi, 2009). Phytosterols have been classified as 4-demethylsterols of the cholestane series, all of which have double bonds at the C-5 position of the ring. In plants, more than 200 different types of phytosterols have been reported the most abundant of which being β-sitosterol, campesterol and stigmasterol (Lagarda *et al*., 2006).

Six sterols in MTSO were determined by Fathi-Achachlouei and Azadmard-Damirchi (2009) at four varieties of MT grown in Iran (2 genetically modified foreign varieties and 2 native varieties).

However, these authors mentioned that there were a few unknown sterols peaks which need further study for identification. In the present study, 15 sterols were determined (see table 8); in addition to the 9 sterols determined by them we could detect, and for the first time , extra 6 sterols .

Results show that the total sterols in cold-pressed MTSO had a higher content (2520mg/kg of oil) compared with four varieties of MTSO (refined solvent-extracted) grown in Iran (1800-2200μg/g) and with seed oil (refined solvent-extracted) of wild growing MT in Turkey (2261mg/kg) (Deraz and Bayram, 1995). Though there was a slight

difference in the values obtained, β-sitosterol was the predominant followed by delta-7-stigmasterol, which in turn is in agreement with the results of four varieties of MTSO grown in Iran and with wild growing MT in Turkey. The high content of β-sitosterol in seed oils (including MTSO) is an additional advantage of these seeds oils.

The difference in total sterols content and values of each sterol between the present study and those studies may be due to the refining process and the method of oil extraction that affect the quality of oil and subsequently the chemical components, including phytosterol content in oil (Lagarda *et al*., 2006) .

Squalene is an important hydrocarbon present in unsaponifiable matter in most oils, mainly olive oil with a reported range of 2500 to 9250 ppm. It is a precursor of phytosterols in vegetable oils (Tuberoso *et al*., 2007; Bail *et al*., 2008). Antioxidant activity of squalene is demonstrated against PUFA (Dessi *et al*., 2002), and it is secondary to that of phenols and tocopherols (Tuberose *et al*., 2007; Nyam *et al*., 2009).

In general, there is a scarcity of information regarding the content of squalene in seed oils. To our knowledge, the present study is the first to provide squalene analysis of MTSO.

Table (8) indicate that the squalene level in MTSO is relatively low (9.35 mg/kg) in comparison with data of other seed oils, where Tuberoso *et al*. (2007) reported a squalene content in sunflower, rapeseed and pumpkin seed oils of 170.5, 437.4 and 3529.9 mg/kg, respectively; a higher content was found in olive oil (5990 mg/kg). Also, they mentioned that squalene was not detected in flax seeds, grape seeds and soybean oils. Maguire *et al*. (2004) also reported in another study that soybean oil and walnut oil had 220 μg/g and 9.4 μg/g of squalene respectively.

Nyam *et al*. (2009) reported that the amount of squalene varies from 36.9 to 1603.2mg/kg in Kenaf and Kalahari melon seed oil, respectively and reached the highest amount in pumpkin seed oil (5907.0 mg/kg).

The decrease in squalene level with ripening of seed oils could be due to an internal mechanism in the seeds that squalene is a biochemical precursor of phytosterol in vegetable oils which in turn is affected by the degree of seed ripening levels before harvesting. That is if seeds are premature or at the beginning of ripening, the level of squalene increases gradually till being fully ripen (proper harvesting date) then the level gradually decrease due to its involvement in phytosterol synthesis. Rodrigues-Estrada (2000) found that oil samples extracted from olives harvested at different ripening levels, had a varying level of squalene and that squalene was practically absent in the oils obtained from mature olives.

1-5 Antioxidant compounds and capacity of cold-pressed MTSO

∝-tocopherol and polyphenols in MTSO were identified as possibly being the main compounds responsible for their free radical scavenging capacity in vegetables oils and foods as well as in the body (Bozan and Temelli, 2008; Nyam *et al*., 2009). In the present study, the result obtained for the amount of ∝-tocopherol content (237.4mg/kg of MTSO) was within the range of previously published results, reported for four varieties of MTSO grown in Iran with values of 187.4 to 465.1 mg/kg (Fathi-Achachlouei and Azadmard, 2009). This wide range of ∝-tocopherol in these varieties may be due to different farming processes. The CN-seed and Budakalaszi varieties produced by irrigated farming generally had lower amount of ∝-tocopherol, compared with other varieties, Babak castle and khoreslo, produced by dry farming which had higher amount of α- tocophers. It is worth mentioning that the MT studied in the present study grows wild not cultivated. On the

other hand, Parry *et al*. (2006) found that cold-pressed MTSO had a lower amount of ∝-tocopherol (156.3mg/kg oil) compared with the present study.

When compared with other oils, ∝-tocopherol amount (237.4 mg/kg of MTSO) was higher than or comparable with those reported for commercial extra virgin olive, peanut, corn and sunflower seed oils (164-578 mg/kg), and higher than soybean oil (92 mg/kg) (Cabrini *et al*., 2001), but much lower than that of wheat germ oil (1330 mg/kg oil) (Wagner *et al*., 2004). They are also higher than those detected in cold-pressed blueberry, red raspberry, marionberry and boysenberry seed oils (21-150 mg/kg oil) (Parry *et al*., 2005).

The total phenolic content (TPC) value of the cold-pressed MTSO (1.16 mg GAE/g oil) was lower than cold-pressed MTSO (3.0 mg GAE/g oil) in the study of Parry and Collegues (2006). Also, it was lower than that of 1.73-2.0 mg GAE/g oil for the cold-pressed red raspberry, blue berry and boysenberry seed oils and that of 1.8-3.4mg GAE/g oil for the cold-pressed parsley, onion, cardamom and mullein seed oils (Parry *et al*., 2005, 2006). The TPC value was greater than of 0.38-0.79mg GAE/g detected in eight Maryland-grown soft wheat grain samples (Moore *et al*., 2005) and that of 0.08-0.70 mg GAE/g oil for the peanut, hazelnut, almond, walnut and pistachio nut oils (Arranz *et al*., 2008). On the other hand, the TPC value was comparable to that of 0.99 and 1.31mg GAE/g oil for the cold-pressed roasted pumpkin and black cumin seed oils, respectively (Parry *et al*., 2006; Lutterodt *et al*., 2010). The differences in TPC may be explained by the different extraction solvents which were used during sample preparation in these studies. 100% methanol is a preferred solvent for antioxidant extraction compared with 50% acetone or 70-80% (methanol: water) solution. 100% methanol is an excellent solubilization of lipophilic antioxidants. The effects of solvent extraction on antioxidant compounds

estimation were observed and discussed in details in the study of Parry and Collegues (2006).

On the other hand, Gorinstein *et al*. (2003) and Tuberoso *et al*. (2007) explained that these differences were due to genetic factors variation, growing conditions such as soil and temperature, post-harvesting treatments including the mechanical grinding during oil processing and storage may significantly alter the chemical composition of selected botanical materials. To assess the antioxidant capacity, the two most common radical scavenging assays DPPH and ABTS radical were chosen because they are widespread methods used and based on an electron transfer and involves reduction of colored oxidants (Apake *et al*., 2007; Niki, 2010).

The cold-pressed MTSO methanolic extract exhibited $DPPH^{\bullet}$ scavenging capacity at IC_{50}=3.34 mg/ml indicating a higher scavenging capacity than the result obtained by Parry *et al* (2006); who got an IC_{50} of 6.7 mg/ml as they were comparing MTSO with other seed oil methanolic extracts. Parsley seed oil exhibited the strongest $DPPH^{\bullet}$ scavenging capacity and IC_{50} of 0.91 mg/ml, onion seed oil extract had the next strongest $DPPH^{\bullet}$ scavenging capacity (IC_{50} of 2.3 mg/ml), followed by Cardmom and roasted pumpkin seed oil extract with IC_{50} = 5.8 and 6.4 mg/ml, respectively.

In other studies, water-soluble extracts of cardamom seed and whole parsely were examined for their IC_{50} against DPPH radical. The cardamom seed extract demonstrated an IC_{50} = 7.8mg/ml while the IC_{50} of the whole parsley extract was 12mg/m (Hinneburg *et al*. 2006). This difference may be due to the fact that methanolic extract had higher phenolic compounds as a result of extraction of both non polar and semi polar soluble phenolic acids.

When comparing all previously mentioned extracts with ascorbic acid, BHA, BHT and gallic acid as references potent antioxidants for DPPH radical scavenging capacity, the

IC_{50} values were 0.09, 0.09, 0.21 and 0.03mg/ml, respectively (Garjani *et al*, 2009; Lutterodt *et al*., 2010).

Regarding the TAC (ABTS assay) the cold-pressed MTSO had a higher TAC value (2.29mmol/L) compared with other methanolic extracted seed oils such as flaxseed, grape, peanut, pumpkin, rapeseed and sunflower seed which had TAC values of 1.01, 1.43, 0.45, 0.95, 0.80 and 1.11 mmol/L respectively. On the other hand, the TAC value in the present study was comparable with maize and soybean oil (2.3 and 1.85 mmol/l respectively) (Tuberoso *et al*., 2007; Bail *et al*, 2008).

There was a difference between values obtained from the two used assays. This is because the TAC (ABTS assay) is based on the generation of a blue/green $ABTS^{\bullet+}$, which is applicable to both hydrophilic and lipophilic antioxidant systems; whereas DPPH assay uses a radical dissolved in organic media and is, therefore, applicable to hydrophobic systems, This finding is in agreement with what was explained by Floegel *et al*. (2011).

2- Animal Experimentation

There are few data on the evaluation of the effect of milk thistle and /or seeds on blood lipids and total antioxidant capacity in blood. The aim of these studies was to demonstrate a pharmaceutical effect of MTS as based on the use of its extract, derivatives, or whole seed poweder. Skottova *et al*. (2003) and sobolova *et al*. (2006) conducted short term studies (18 day and 21 day, respectively) to examine the effect of polyphenotic fraction of silymarine on lipoprotein profile in rats.

To our knowledge, no studies were conducted to investigate the effect of the milk thistle seed oil on blood lipids and total antioxidant capacity.

To achieve better results, the American Institute of Nutrition Rodent Diets (AIN-93), was adopted in the present study. The AIN-93 diet proved to give a better balance of essential nutrients for long-term studies (Reeves, 1997). For example, soy bean oil was used as an adequate source of essential fatty acid, linoleic and linolenic acid. Whether for soybean oil in control group or MTSO, the amount of oil in the present study was added to reach the maintainance requirements of adult rats (4% of the diet) according to Reeves (1997). Additional two groups of soybean oil and MTSO with oil of 8% were added to exsert the effect of the tested oil on blood lipids, TAC and MDA.

2-1 Body weight, liver weight, accumulative food intake and FER:

The results of the present long-term MTSO feeding study (12 weeks) show non significant change in final body weights, body weight gain of rats feed two levels of MTSO compared with control diet groups (table 10). These results were consistent with those of many animal studies on different seed oils (Moon *et a*l., 2001; Gorinstein *et al*., 2003; Cintra *et al*., 2006; Makni *et al*., 2009; Visavadia *et al*., 2008). However, liver weights were significantly different in 8% MTSO and 8% control of cholesterol supplemented diet. Compared to other diet groups. An obvious increase in liver size

(hepatomegaly) was particularly observed in groups fed cholesterol supplemented diet in the present study. It appears that the high-cholesterol diet caused an increase of liver weight. This may be due to accumulation of lipids in liver because no difference in body weight was observed across rats in different groups. This was in accordance with the finding of Ha et al. (2005) who studied the effect of rice bran oil on lipid profile in Sprague- Dawley rats fed high cholesterol diet (1%) for 4 weeks. On the other hand, Visavadiya and Narasimacharya (2008) did not find any significant differences in liver weight when feeding sesame seed poweder with 0.5% cholesterol for 4- weeks which is lower than that used in present diet (1%).

Accumulative food intake and food efficiency ratio (FER) of rats fed two levels of MTSO were insignificantly different with and without cholesterol (table 12). This is consistent with the results obtained by Ha et al. (2005) who have reported insignificant changes in FER in rats fed rice-bran oil diet with and without cholesterol. This was also confirmed by Cintra et al (2006) who found no significant difference in FER when studying four types of seed oils in rats.

Irrespective of cholesterol content, body weight gain, accumulative food intake and FER were not significantly different. When taking cholesterol into account, liver weights were significantly different in 8% of MTSO and control diet in cholesterol- supplemented diet. Irrespective of cholesterol there become no significant differences in liver weights among all diet groups. This suggest a significant effect of cholesterol deposition in liver and subsequently an increased liver weight (table 10-a). This suggestion is assured when taking the effect of cholesterol and oil % irrespective of oil type where there still a significant difference in liver weight in cholesterol supplemented diets (table 10-b). Irrespective of oil % (table 10-c) liver weight is still significantly different, which

highlighted the effect of cholesterol on liver weight. Reasons will be discussed later with other findings.

2-2 Levels of blood lipids and lipoproteins

The results of the present study show a high significant difference in total cholesterol (TC) in 8% MTSO and 8% control cholesterol- supplemented diets when compared to 8% control cholesterol- free diet. Also LDL-C of cholesterol- supplemented diet groups were significantly higher than cholesterol- free diet groups. An interesting result in the present study is the high levels of HDL-C in rats fed cholesterol – free diet with MTSO 4% and MTSO 8% when compared to cholesterol- supplemented diets. Present results are in accordance with those obtained by Ha *et al*. (2005) who found high and significant differences in the total cholesterol in high cholesterol control diet and rice bran oil supplemented with cholesterol when compared to control groups. Regarding HDL-C they found that feeding rice bran oil with add cholesterol to rats resulted in high levels of HDL-C when compared with cholesterol control group but not higher than cholesterol free-control group which in turn resulted in significantly higher levels of HDL-C than other groups. Similar findings were also observed by Gorinstein *et al*. (2003) when feeding oil-supplemented diets with or without cholesterol to rats. They found that sunflower and rape seed oil with cholesterol- supplemented diets and cholesterol control diet resulted in higher serum total cholesterol and LDL-C when compared with sunflower seed oil or rape seed oil diets. In the same study HDL-C levels in cholesterol - containing diets did not differ from those in control group. Gurr *et al*. (2002) explained that the lipoprotein pattern of rats is of the HDL type; that is, HDL is the dominant lipoprotein class. The function of this large HDL-C is poorly understood and highly variable between species.

On the contrary, safflower seed extract diets supplemented with cholesterol resulted in decreased TC when compared with cholesterol- containing control group (Moon *et al*,

2001). The authors explained that total phenolic content and low saturated fatty acid content in safflower extract were most potent in lowering the plasma cholesterol-concentrations. Also, Moon and Co-workers (2001) came with the same results of Gorienstein *et al* (2003) with respect to HDL-C with no significant differences in safflower seed extract cholesterol diets compared with cholesterol containing group. While the HDL-C/TC ratio was higher for safflower seed extract cholesterol diet than in control group due to decrease in TC.

Similar results were obtained by Makni et al. (2008) who found that flax and pumpkin seed mixture diet supplemented with cholesterol resulted in lower plasma TC and LDL-C when compared with cholesterol - supplemented control diet. Whereas for HDL-C, levels were higher in mixed seeds cholesterol supplemented diets when compared with cholesterol control group. However, results were not similar in all of the studies. The discrepancy might be attributed to the protocols and composition of diet mixtures.

Our results regarding high blood TC and LDL-C at 8 % control and MTSO cholesterol-supplemented diets, compared to 8 % control cholesterol- free diet suggest cholesterol and oil level dose – effects. To elucidate the % of fat, Reeves (1997) recommended that fat % in maintenance diet for adult rats should be at 4% of the diet, giving (a polyunsaturated: saturated fatty acid) ratio of at least 2:1 for soybean oil. This ratio is of great influence on tissue lipids and eicosanoids production.

Fatty acid pattern of MTSO in the present study was found to resemble that of soybean oil (control oil) and subsequently reaching the ratio mentioned above. Soybean oil content of saturated fatty acid (SFA) is 15.9% and PUFA content is 61.5% (Whent *et al.*, 2010) while for MTSO in present study SFA is 19.5 % and PUFA is 57.5%. The SFA'S, palmitic acid (C16:0) and stearic acid (C18:0), are found at 8.6%, 5.2% and 10.9%, 4.9% in MTSO and soybean oil respectively. The relatively high content of both fatty acids in

MTSO and soybean oil may give rise to high blood TC and LDL-C as reported by Salter *et al*. (1998), Billett *et al*. (2000), Kritchevsky *et al*.(2001) and Cintra *et al* (2006). They have clarified that the fatty acid composition of dietary fat is important in determining plasma lipoprotein and cholesterol concentration and thus an interactive effect of palmitic acid and stearic acid as SFA in diet trigger high level of cholesterol in blood. They also found that supplementing experimental diet with different levels of high cholesterol exaggerated blood cholesterol levels and platelete aggregation in rats, which in turn becomes more hypercholesterolaemic. Kritchevsky *et al*.(2001) conducted a study about serum and liver lipids in rats fed various mixtures of corn and palm oils (high level of palmitic acid) with and without cholesterol . They found that introduction of the lowest level of palm oil raised serum cholesterol levels significantly and they continued to rise as more palm oil was introduced into the diet containing cholesterol. They concluded that palmitic acid becomes hypercholesterolemic only in cholesterol containing diets. However, they demonstrated the noncholesterolemic effect of palmitic acid in rats fed cholesterol-free diets. Moreover, in certain rats, hamster and monkey experiments a generalized cholesterolemic effect of saturated fat occurred only if some cholesterol was present in the diet (Gilani *et al*.,2002) .Dietary cholesterol appears to down regulate the LDL-C receptor activity, which would decrease LDL removal and thereby elevate circulating levels of cholesterol.

Billett *et al* (2000) and Cintra *et al*. (2006) suggested an interactive effect between different dietary SFA and dietary cholesterol on lipoprotein metabolism through exerting a specific modulation of the expression of the LDL receptor and apolipoprotein B-genes and subsequently increase of the atherogenicity. These findings are in agreement with the results of present study with respect to TC, LDL-C, HDL-C/LDL-C ratio, TC/TG ratio and atherogenic index (AI) as affected by cholesterol and type of oil and % of oil (table 15 and 16). These results were obviously elucidated when statistically calculated once

irrespective of cholesterol, once irrespective of oil % and once irrespective of oil type (table 17 a, b, c). This confirms that % of oil and presence of cholesterol are the main factors in the present study affecting the lipid profile and calculated AI. To support present study results regarding higher TC and lower HDL-C as atherogenic factors, AI was calculated. There was an obvious and significant higher AI values for cholesterol-supplemented diets at 8% of oil.

With respect to blood TG concentration in present study, no significant variations were found in MTSO diets when compared with control diets whether with or without cholesterol. Although the reasons for- these observations are unclear. One of the possibilities is that the relatively high saturated fatty acid content of MTSO and soybean oil in diet groups could have influenced the TG levels as SFA are known substrates for TG synthesis (Harvey and Champ, 2005). This was clarified by Visavadiya *et al*. (2008) who failed to find any significant variation in blood TG in experimental diets using sesame seeds oil and powder which is high in SFA content. These results are consistent except for the 4% cholesterol- free control diet group which tend to have higher TG value ($p< 0.05$) when compared to all diet groups. Moreover, irrespective of oil type or cholesterol supplementation of diets, TG concentration in present study tends to be obviously higher in cholesterol- free diet at 4% oil in control group. A result that may be explained by the presence of higher carbohydrate content in expense of fat in 4% fat groups when compared with 8% fat-containing groups (table 4). This was in accordance with result found by Cintra *et al* (2006) who found that animal feed normal diet had higher levels of TG than did animals fed high fat diet.

It is worth mentioning in the present study that despite the relatively good content of PUFA (57.5%), phyto-sterols (2520 mg/kg) mainly β-sitosterol (46% of total phytoseroles), α-tocopherols (237.4 mg/kg) and TPC (1.16 mg/g oil) in MTSO, its effect

on blood lipoprotein did not differ, when comparing 4% MTSO with 4% control group and 8% MTSO with 8% control group. This could be due to similar chemical composition of soybean oil used in control diet and examined MTSO.

This was obvious when taking into account both effect of % of oil used and presence of cholesterol in diets irrespective of oil type. At 4% oil in presence of cholesterol values of serum TC, LDL-C, TC/TG and AI were significantly lower than values at 8%.This may be due to presence of bioactive components (phytochemical compounds) in MTSO and control oil(soybean oil) and lower concentration of SFA at 4% in comparison with 8% that could lead to inhibition of HMG-CoA reductase and thus reduction of blood lipid indicators. However, it is well-known that HMG-CoA reductase inhibitors drugs (lovastatine) do not have a hypocholesterolemic effect in rodents (rats) yet do in hamsters, rabbits and humans; this might be due to differences in lipoprotein metabolism among animal species as reported by Bok *et al*. (1999). They mentioned that despite these differences rats can be used as an animal model to study cholesterol metabolism if experimental conditions are appropriately controlled. Doses of HMG-CoA reductase inhibitors have to be very high to exert a hypocholesterolemic response in rats. Bok *et al.* (1999) found that tangerine peel extract and a mixture of two citrus flavonoids (naringin and hesperidin) when supplemented with cholesterol (1%) lowered plasma lipid profile of Sprague-Dawely rats significantly when compared cholesterol – control group. They explained that highly concentrated citrus bioflavonoids have inhibited the measured HMG-CoA reductase and thus have decreased plasma lipid levels.

In the present study increasing the % of tested MTSO and control soybean oil to 8% as hypothesized to decrease serum lipids, did not result in lowering serum lipids. On the contrary values were significantly higher than 4% of oil diets supplemented with cholesterol. The expected positive effect of different phytochemical compounds was not

observed at 8% oil this may be due to higher SFA concentration in 8 % oil which in turn may hinder the effect of different phytochemical compounds in lowering blood lipids.

2-3 Lipid peroxidation and total antioxidant capacity in blood:

MDA is a breakdown product of spontaneous fragmentation of peroxides form from polyunsaturated fatty acids (PUFA) mainly from the oxidation of cell membranes (Niki, 2010).

In the present study, the measurement of lipid peroxidation in serum, expressed as MDA (nmol/ml), showed clearly high levels of MDA in 8% cholesterol- free control diet when compared with other cholesterol- free diets. Additionally the 8% cholesterol supplemented control diet was higher than that of 8% cholesterol supplement MTSO diet ($P<0.05$) (table 19). Irrespective of cholesterol (table 17a) MDA level was high in 8% control oil in comparison with other diet groups. While in the presence of cholesterol at 4% and 8% fat % MDA levels were higher when compared with 4% without cholesterol (table 17b). When exploring the effect of oil type with cholesterol (irrespective of fat %) MDA was also high in control oil with or without cholesterol (table 17c). This shows that higher level of MDA was in 8% soybean control oil with or without cholesterol. This is because soybean oil contains high amount of unsaturated fatty acids and lower SFA and is more susceptible to oxidation when compared with MTSO which is lower in oxidation. This was explained by Augilera *et al*, (2004) and Nevin *et al*, (2008), who found that feeding oil rich in PUFA and has low antioxidant activity results in their accumulation of MDA in cell membranes and increased the oxidative stress, since PUFA's are highly susceptible to peroxidation compared to MUFA and SFA.

This may give a reason for the higher MDA formation in the serum of rats fed soybean control oil which contain low α – tocopherol level and high PUFA's. Moreover,

Niki (2010) stated that MDA concentration in serum are inversely correlated to the proportion of PUFA mainly linoleic and linolenic acid in serum lipoprotein lipids. His findings suggested that other factors such as the availability of antioxidants, polyphenols, unsaponifiable compounds (such as phytosterols and squalene) may be of greater influence on intravascular lipid peroxidation.

In the present study MTSO rich in linoleic acid (56.8% n-6), minor amount of linolenic acid (0.76% n-3), good amounts of MUFA (22%) and relatively high amounts of SFA (19.5%) is compared to control oil (soybean oil). Soybean oil is rich in linolenic acid (7.5%), linoleic acid (54%), MUFA (22.7%) and SFA (15.9%) (Whent *et al*., 2010). We cannot ignore the presence of phytochemical compounds in the experimental MTSO which is nearly similar to control oil (soybeanoil) except for α – tocopherol, where MTSO has higher (237.4 ppm) than soybean oil (92.1 ppm).

Baba et al. (2000) showed that sesame oil contains small amounts of tocopherols but has good amounts of other phenols which makes it a unique oil because of its superior oxidative stability. Olive oil has the biophenol which was shown to improve *in vivo* an antioxidant defences (Agulira et al., 2004). On the other hand, canola oil, although rich in oleic acid (62.5%), has low content of saturates and of tocopherols which are removed during the refining process, and its high content of α- lenolenic (n3) (≈ 10%) may render it susceptibile to lipid peroxidation (Baba *et al*. 2000). In one study, Fang *et al* (2004) found that genistein (an isoflovonoid from soy) raised the serum total antioxidant capacity TAC and decreased the MDA.

In the present study, when taking into consideration the effect of high cholesterol diet, there were high lipid peroxidation of MDA (12.2 nmol/ml) for 8% soybean control oil diet group with cholesterol. The MDA levels were exaggerated due to the effect of both cholesterol and oil type since soybean oil is high in PUFA. These results corresponded

with the observations that cholesterol supplemented diet leads to a decrease in blood antioxidant activity and increase in MDA (Gorinstein *et al*., 2003). This effect was focused on by Gokkusu *et al* (2004) who found that high cholesterol diets supplemented with polyunsaturated fats seem to have a tendency to exaggerate lipid peroxidation in serum, as well as to disturb the balance between cholesterol and phospholipid in blood. Recently, Nevin and Rajamohan (2008) studied the effects of feeding virgin coconut oil (VCO) of low PUFA on *in vitro* oxidation of LDL in comparison with copra oil of high SFA and sunflower oil of high PUFA in cholesterol fed rats. VCO was found to prevent the oxidation of LDL from oxidants. The properties of VCO may be attributed to the presence of biologically active unsaponifiable phytochemical components (α-tocopherols, polyphenols and phytosterols) which are more active than other tested oil.

Regarding the total antioxidant status, TAC was higher in 4% cholesterol free MTSO diet when compared to 8% cholesterol free control diet (soy oil) and 4% cholesterol supplemented MTSO diet. Irrespective of oil % higher antioxidant status was found in cholesterol - free MTSO diets. The TAC result was not surprising since the MDA value of 4% MTSO cholesterol- free diet was low when considering the type of oil. It seems the TAC obtained here is attributed to the presence of phenolic compounds, phytosterols mainly β- sitosterol (46%) and α- tocopherol, a potent antioxidant, in MTSO. The result of serum TAC in MTSO group was on going with those of *in vitro* TAC values of MTSO determined in this study. The TAC value of MTSO oil was 2.29 mmol/l which is considered high when compared to TAC of other oils. TAC values of soybean oil is 1.75 mmol/l, olive oil 1.29 mmol/l, flaxseed oil 1.01 mmol/l and sunflower oil 1.1 mmol/l as reported by Tuberose *et al* (2007). TAC value of corn oil (2.3 mmol/l) was similar to that of MTSO (Cintra *et al*.,2006).

Bartosz (2003) pointed that the long term dietary intervention may not result in significant changes of serum TAC as compared with control groups. He has reported that oral supplementation of ascorbic acid for 4 weeks did not affect TAC significantly when compared to control group. He has also reported the same effect after 30 days of feeding low- birth- weight infants with a formula containing w6 and w3 long chain PUFA as compared with a group fed human milk. The author explained that there is a possible contribution of polyphenols present in blood plasma at the concentrations of 0.2-2 mM. However, this may explain the lack of significant differences in TAC values in different diet groups of the present study.

Conclusions

Under the conditions of this study, the following can be concluded:

- Fat represented the major component in MTseeds (26.9%). Cold-pressing technique resulted in high quality MTSO with low acidity and peroxide value as quality parameters.
- Cold-pressed MTSO could have a long shelf-life with greater OSI value at 80 Ċ which was higher than commercial soybean oil and it was lower than commercial corn oil. These may be due to the ratio of USFA: SFA, amount of polyphenolic compounds, α-tocopherols and other natural – occurring antioxidant in the MTSO.
- Among the 13 fatty acids detected in MTSO in this study, the main fatty acids were found to be linoleic acid, oleic acid, palmitic acid and stearic acid; linolenic acid was found in minor amount.
- Cold-pressed MTSO had high content of phytosterols (mainly B-sitosterol) and α-tocopherol which are considered important from a nutritional and functional point of view. However, squalene was found in a relatively low level.
- Cold-pressed MTSO was found to have a high *in vitro* free radical scavenging capacity as indicated by TAC due to the presence of polyphenolic compounds and α- tocopherol.
- Feeding rats cholesterol-free or cholesterol-supplemented diets with two levels of experimental MTSO and control oil resulted in similar final body weights and body weight gain
- Liver weights were significantly higher in rats fed 8% MTSO and 8% control cholesterol- supplemented diets. Irrespective of oil type, liver weights in 8% cholesterol- supplemented diet groups were significantly higher than other groups.

- A significant higher TC value was found in cholesterol- supplemented 8% MTSO and control oil diet groups compared with 8% control cholesterol-free diet.
- Serum LDL-C levels of rats fed cholesterol –free diet were significantly lower than those fed cholesterol –supplemented diets. Feeding 8% MTSO and 8% control oil with cholesterol resulted in higher serum LDL-C level.
- Serum HDL-C levels tended to be the highest in cholesterol -free MTSO diet groups. Lower HDL-C values were obtained in all cholesterol-supplemented diet groups. Consequently, HDL/LDL values were significantly lower in cholesterol-supplemented diet groups compared to cholesterol-free diet groups.
- TC/TG ratio and Atherogenic index (AI) were found to be significantly higher at 8% MTSO and 8% control oil cholesterol-supplemented diet groups compared to other diets without cholesterol.
- Irrespective of cholesterol, TC, LDL-C, TC/TG ratio, HDL-C, HDL-C/LDL-C ratio and AI values were not significantly different in all diet groups, while irrespective of oil type, TC, LDL-C, TC/TG ratio and AI were significantly higher at 8% cholesterol –supplemented diets.
- TG levels were significantly higher in cholesterol-free 4% control oil diet compared to cholesterol –free 4% MTSO diet group
- TAC value of 4% MTSO-cholesterol free diet was significantly higher compared to 4% MTSO- cholesterol supplemented diet group. Otherwise, no differences were found. Irrespective of cholesterol and irrespective of oil type, TAC value was the same in all diet groups, while irrespective of oil % (effect presence of cholesterol and oil type), TAC was higher at MTSO cholesterol-free diet.
- MDA was significantly lower in 8% MTSO cholesterol-supplemented diet and 8% MTSO and 4% MTSO in cholesterol-free diet groups compared to 8% control diet

in both cholesterol-supplemented and cholesterol-free groups. Irrespictive of cholesterol, MDA level at 4 % and 8 % MTSO was significantly lower than control oil at 8 %, while irrespective of oil %, MDA was found to be significantly higher at control oil diet with and without cholesterol compared to MTSO diet in both cholesterol-supplemented and cholesterol-free groups.

Recommendations

- Further comprehensive studies are needed to compare MTSO with other seed oils like sunflower, safflower and black cumin with respect to fatty acid profile and bioactive compounds and its effect on serum, liver and adipose tissue lipid profile.
- Analysis of total cholesterol, lipoproteins, enzyme activities in liver and investigating histopathological effects of MTSO lipids on liver tissue are also recommended.
- Other animal models should be considered in future studies to evaluate the effects of different lipid sources in context of a high cholesterol diet and to elucidate the mechanisms by which the diet may modulate lipid profile.
- Study of fatty acid composition, antioxidant properties, and anti proliferative capacity of cold-pressed Milk- Thistle seed cake (a by-product of cold- pressed seed oil).

References

Abolfazl, F., Barzegar, M. and Azizi, M. H. (2006). Determination of fatty acids and total lipid content in oil seed of 25 pomegranates varieties grown in Iran. **Journal of Food Composition and Analysis**, 19, 676-680.

Abu-Jadayil, S., Tukan, S.K. and Takruri, H.R (1999). Bioavailability of iron from four different local food plants in Jordan. **Plant Foods for Human Nutrition**, 54, 285-294.

Abu-Rajouh, K. S. (1996). A study of the Nutritive value of milk thistle seeds *Silybum marianum* L. Gaertn. **Unpublished Master Dissertation**, university of Jordan, Amman, Jordan.

Abu-Rajouh, K.S. and Takruri, H.R. (2000). Studing the nutritional value of milk thistle seeds. **Arab Journal for Food and Nutrition**, 2 (Aug.) 31-47. (In Arabic).

Aguilera, C.M., Mesa, M.C., Tortosa, M.C., Nestares, M.T., Ros, E. and Gil ,A. (2004). Sunflower oil does not protect against LDL oxidation as Virgin olive oil does in patients with peripheral vascular disease. **Clinical Nutrition**, 23, 673-681.

Ahotupa, M. and Vasankari, T.J. (1999). Baseline diene conjugation in LDL lipids: An indicator of circulating oxidized LDL. **Free Radical Biology and Medicine**, 27,1141-1150.

Al- Eisawi, D.M. and Takruri, H.R. (1989). A checklist of wild edible plants in Jordan**.** **Arab Gulf Journal for Science Research Agriculture and Biological Science**, B7 (1), 79-102.

Al-Ismail, K., Herzallah, S.M. and Rustom, A.S.(2007). Antioxidant activities of some edible wild Mediterranean Plants. **Italian Journal of Food Science**, 19 (3), 287-296.

American Dietetic Association and Dietitians of Canada (2007): Dietary fatty acids. **Journal of the American Dietetic Association**, 107 (9), 1599-1612.

American Heart Association (2006). Diet and Lifestyle Recommendations Revision 2006: a scientific statement from the American Heart Association Nutrition committee. **Circulation**, 114(1), 82-96.

Antolovich, M., Prenzler, P.D., Patsalides, E., Mc Donald S. and Robards, K. (2002). Methods for testing antioxidant activity. **Analyst**, 127, 183-198.

AOAC. (2000). **Official Methods of Analysis of the Association of Official Analytical Chemists,** (18th ed.). USA, Virgina: Association of Official Analytical chemists.

Apak, R., Guclu, K., Demirata, B., Ozyurek, M., Celik, S.E., Bektasoglu, B., Berker, K.I. and Ozyurt, D. (2007). Comperative evaluation of Various total antioxidant capacity assays applied to phenolic compound with the CUPRAC Assay. **Molecules**, 12, 1496-1547.

Arena, E., Campisi, S. Fallico, B. and Maccarone, E. (2007). Distribution of fatty acids and phytosterols as a criterion to discriminate geographic origin of pistachio seeds. **Food Chemistry**, 104, 403-408.

Arranz, S., Cert, R., Jimenez, J. and Calixto, F. (2008). Comparison between free radical scavenging capacity and oxidative stability of nut oils. **Food Chemistry**, 110, 985-990.

Arranz, S., Cert, R., Perez- Jamenez, J., Cert, A. and Saura- Calixto, F. (2008). Comparison between free radical scavenging capacity and oxidative stability of nut oils. **Food Chemistry**, 110, 985-990.

Baba, N.H. Ghossoub, Z. and Habbal, Z. (2000). Differential effects of dietary oils on plasma lipids, lipid peroxidation and adipose tissue lipoprotein lipase activity in rats. **Nutrition research**, 20 (8), 1113-1123.

Bail, S., Stuebiger, G., Krist, S., Untrwager, H. and Buchbaner, G. (2008). Characterization of Various grape seed oils by volatile compounds, triacylghyceral composition, total phenols and antioxidant capacity. **Food Chemistry**, 108, 1122-1132.

Barreto, J.F.A, Wallace, S.N., Carrier, D.J. and clausen, E.C. (2003). Extraction of Nutraceuticals from milk thistle 1. Hot water extraction. **Applied Biochemistry and Biotechnology**, 105-108, 881-889.

Bartosz, G. (2003). Total Antioxidant Capacity. **Advance in Clinical Chemistry**, 37, 219-292.

Bergmann, K.V., Sudhop, T. and Lutjohann, D. (2005). Cholesterol and Plant Sterol absorption: Recent Insights. **American Journal of Cardiology**, 96 (suppl.), 10D-14D.

Besbes, S., Blecker, C., Deroanne, C., Bahloul, N., Lognay, G. and Drira, N. E. (2004). Date seed oil: Phenolic, tocopherols and sterol profiles. **Journal of Food Science**, 11, 251-265.

Bhupathiraju, S.N and Tucker, K.L. (2011). Coronary heart disease prevention: Nutrients, foods, and dietary patterns. **Clinica Chimica Acta**, 412, 1493-1514.

Bianco, A., Coccioli, F., Guiso, M. and Marra, C. (2001). The occurrence in olive oil of a new class of phenolic compounds: Hydroxy- Isochromans. **Food Chemistry**, 77, 405-411.

Billet, M.A., Bruce, J.S., White, D.A., Bennett, A.J. and Salter, A.M. (2000). Interactive effects of dietary cholesterol and different saturated fatty acids on lipoprotein metabolism in the hamster. **British Journal of Nutrition**, 84, 439-447.

Blekas, G., Vasilakis, Harizanis, C., and Boskoy (2002). Biophenols in table olives. **Journal of Agricultural and Food Chemistry**, 50, 3688-3692.

Bok, S.H, Lee, S.H, Park, 4.B., Bae, K.H., Son K.H. Tae- Sook, J. (1999). Plasma and Hepatic cholesterol and Hepatic activities of 3-Hydroxy-3- methy1- glytaryl- COA reductase and Acyl COA: cholesterol transferease Are lower in rats fed citrus peel extract or a mixture of citrus bioflarvonoids. **Journal of Nutrition**, 129, 1182-1185.

Bozan, B. and Temelli, F. (2008). Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. **Bioresource Technology**, 99, 6354-6359.

Brash, A.R. (1999). Lipoxygenases: occurrence, functions, catalysis and acquisition of substrate. **Journal of Biological and Chemical**, 274, 2379-2382.

Brown, D.W., Mokdad, A.H., Walk, H., As'ad, M., Al-Nsour, M. (2009). Projected Burden of Chronic, Non Communicable Diseases in Jordan. **Preventing Chronic Disease**, 6(2), 1-3.

Bulur, H., Ozdemirler, G., OZ, B., Toker, G. and Uysal, M. (1995). High cholesterol diet supplemented with sunflower seed oil but not olive oil stimulates lipid peroxidation in plasma, liver and aorta of rats. **Nutritional Biochemistry**, 6, 547-550.

Cabrini, L.V., Barzanti, M., Capollone, D., Fiorentini, G., Grossi, B., Tolomelli, L, Zambonin, L. and Landi, L. (2001). Antioxidants and total proxyl – radical- trapping ability of olive and seed oils. **Journal of Agricultural and Food Chemistry**, 49, 6026-6032.

Cintra, D.E .C., Costa, A.G.V., Peluzio, M.CG., Matta S.L.P., Silva, M.T.C and Costa, N.M.B. (2006). Lipid profile of rats fed high fat diets based on flaxseed peanut, trout or chicken skin. **Nutrition**, 22, 197-205.

Del Rio, D., Stewart, A.J. and Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. **Nutrition, Metabolism and Cardiovascular Diseases**, 15, 316-328.

Denke, M.A. (2006). Nutrient and Genetic Regulation of lipoprotein metabolism. In: Shils, M., Shike, M., Ross, A. C., Caballero, B. and Cousins, R. (editors), **Modern Nutrition in Health and Disease**, (10th ed.), part V. (PP. 1067- 1074), Philadlphia: lippincott Williams and Wilkins.

Deraz, S., Bayram, E. (1995). Evaluation of chemical contents of medicinal plant (*Silybum. marianum*) wild growing in Turkey. **Ege- Ueniversitesi –Ziraat- Fakueltes Dergisi**, 32, 79-85. (**Abstract**).

Dessi, M.A., Deiana, M., Day, B.W., Rosa, A., Banni, S. and Corongiu, F.P. (2002). Oxidative stability of polyunsaturated fatty acids: effect of squalene. **European Journal of Lipid Science and Technology**, 104, 506-512.

Dimitrios, B. (2006). Sources of natural phenolic antioxidants. **Trends in Food Science and Technology,** 17, 505-512.

European committee. (2003). On the characteristics of Olive oil and olive residue oil and on the relevant methods of analysis. CONSLEG: 199 IR258-01/11/2005. **Office for Official puplications of th European Communities**.

Fang, Y. C., Chem, B.H, Hung, R.F and Lu, Y.F. (2004). Effect of genistein supplementation on tissue genitin and lipid peroxidation of serum, liver and low-density lipoprotein in hamsters. **Journal of Nutritional Biochemistry**, 15, 142-148.

Fang, Y. Z, Yang, S. and Wu, G. (2002). Free radicals, antioxidants, and nutrition. **Nutrition**, 18, 872-879.

Fathi- Achachlouei, B. and Azadmard- Damirchi, S. (2009). Milk thistle seed oil constituents from different varieties grown in Iran. **Journal of American Oil and Chemical Society**. 86, 643 – 649.

Finaud, j., Lac, G. and Filaire, E. (2006). Oxidative stress: relationship with exercise and training. **Sports Medicine**, 36, 327-358.

Floegel, A., Okkim, D., Chung, S.J., Koo, S.I. and Chun, O.K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant- rich US foods. **Journal of Food Composition and Analysis**, 24, 704-711.

Garjani, A., Fathiazad, F., Zakheri, A., Akbari, N.A., Azmarmie A.F., Andalib, S. and Maleki- Dizaji, N. (2009). The effect of total extract of *Securigera Securidaca* L. seeds on serum lipid profiles, antioxidant status and vascular function in hypercholesterolemic rats. **Journal of Ethnopharmacology**, 126, 525 – 532.

Gershman, R. and Fenn, W.O. (2001). Oxygen Poisoning and x-irradiation: a mechanism in common. 1954. **Nutrition**, 17, 162-169.

Gilani, G.S., Nimal Ratnayake, W.M., Brooks, S. P.J., Botting, H.G., Plouffe, L.J. and Lampi, B.J. (2002). Effect of dietary protein and fat on cholesterol and fat metabolism in rats. **Nutrition Research**, 22, 297-311.

Gimeno, E., Castellote, R.M., Torre, M.C. and Lopez-Sabater, M.C. (2000). Rapid determination of vitamin E in vegetable oils by reversed phase high performance liquid chromatography. **Journal of Chromatography** A, 881, 251-255.

Gokkusu, C., Ozden, T.A and Mostafa, T. (2004). The relationship between oxidative stress and membrane ATP ase enzyme activities in hypercholesterolemic rats supplemented with vitamin. **European Journal of Medicine**, 4, 70-76.

Gorinstein, S., Leontowicz, H., Lojek, A., Ciz, M., Krzminski, R., Zachwieja, Z., Jastrzebski, Z., Delgado- Licon, E., Martin Belloso, O. and Trachtenberg, S. (2003). Seed oil improve lipid metabolism and increase antioxidant potential in rats fed diets containing cholesterol. **Nutrition Research** 23, 317-330.

Grundy, S.M. (2006). Nutrition in the management of Disorders of Serum Lipids and lipoproteins. In: Shils, M., Shike, M., Ross, A.C, Caballero, B. and Cousins, R. (editors), **Modern Nutrition in Health and Disease**, (10^{th} ed.), part V. (PP.1076-1094), Philadelphia: lippincott Williams and Wilkins.

Gurr, M.R., Harwood, J.L. and Frayn, K.N (2002). **Lipid Biochemistry**, (5^{th} ed.). London: Blackwell Science.

Ha, T.Y., Han, S., Kim, S.R, Kim, I.H, Lee, H.Y. and Kim, H.K. (2005). Bioactive components in Rice bran oil improve lipid profiles in rats fed a high- cholesterol diet. **Nutrition Research**, 25, 597-606.

Hammoudeh, A.J., Izraiq, M, Al-Mousa, E., Al- Tarawneh, H., El-Harassis, A., Mahadeen, Z., Badran, N. and Haddad. J. (2008). Serum lipid profiles with and without CAD: Jordan hyperlipidaemia and related targets study (JOHARTS-1). **Eastern Mediterranean Health Journal**, 14 (1) 24-32.

Harvey, R.A., Champe, P.C. and Ferrier, D.R. (2005). **Biochemistry**, (3^{rd} ed). Philadelphia, USA, Lippincott Williams and Wilkins.

Hinneburg, I., Dorman, H.J.D. and Hiltunen, R. (2006). Antioxidant activities of extracts form selected culinary herbs and spices. **Food Chemistry**, 97, 122-129.

Houenschil, A., Bretzel, R., Hardt, P.D and Ewald, N. (2010). Successful treatment of severe hypertriglyceridemia with a formula diet rich in omega-3 fatty acids and medium-chain triglycerides. **Annual Nutrition Metabolism**, 56, 170-175.

Jacobs, B.P., Dennehy, C., Ramirez, G. and Lawrence, V. A (2002). Milk thistle for the treatment of liver disease: A systematic review and meta-analysis. **The American Journal of Medicine**, 113, 506-515.

Jentzsch, A.M., Bachmann, H., Furst, P. and Biesalski, H.K. (1996). Improved analysis of malondialdehyde in human body fluids. **Free Radical Biology and Medicine,** 20 (2). 251-256.

Joint FAO/WHO Food Standards Program, Codex standards for named vegetable oils, Codex- STAN 210, Amended 2005.

Kamal- Eldin, A., Appelquist, L.A. and Yosif, G. (1994). Lignan analysis in seed oils from four sesamum species: comparison of different chromatographic methods. **Journal of the American Oil Chemists Society**, 71, 141-145.

Katiyar, S. (2005). Silymarin and skin cancer prevention: anti- inflammatory, antioxidant, and immunomodulatory effects. **International Journal of Oncology**, 26,169.

Kattan, M.B., Zock, P.L. and Mensink, R.P. (1994). Effects of fats and fatty acids on blood lipids in humans: an overview. **American Journal of Clinical Nutrition**, 60 (supp.), 1017S- 1022S.

Khader, Y.S., Batieha, A., Al-Khateeb. M., Omari, M., and Ajlouni, K. (2010). Prevalence of dyslipidemia and its associated factors among Jordanian adults. **Journal of Clinical Lipidology**, 4, 53-58.

Knekt, P., Kumpulainen, J., Jorvinen, R., Rissanen, H., Heliovaara, M., Reunanen, A., Hakulinen, T. and Aromaa, A. (2002). Flavonid intak and risk of chronic diseases. **American Journal of Clinical Nutrition**, 76, 560-568.

Knight, J.A. (2000). The biochemistry of aging. **Advances in Clinical Chemistry**, 35, 1-62.

Kritchevsky, D. and Chen, S. (2005). Phytosterols- health benefits and potential concerns: a review. **Nutrition Research**, 25, 413-428.

Kvasnick, F., Biba, B., sevcik, R., Voldrich, M. and Kratka, J. (2003). Analysis of the active components of silymarin. **Journal of Chromatography A**, 990, 239-245.

Lagarda, M.J., Garcia, G.L. and Farre, R. (2006). Analysis of Phytosterols in foods. **Journal of Pharmaceutical and Biomedical Analysis**, 41, 1486-1496.

Lapenna, D., Ciofani, G., Pierdomenico, S.D. and Cuccurullo, F. (2001). Reaction Conditions affecting the relationship between thiobarbituric acid reactivity and lipid peroxides in human plasma. **Free Radical Biology and Medicine**, 31 (3), 331- 336.

Lee, J.K., Min, D.B. (2004). Reactive oxygen species, Aging and antioxidative Nutraceuticals. **Comprehensive Review of Food Science and Food Safety**, 3, 21-33.

Luttrodt, H., Luther, M., Slavin, M., Yin, J., Parry, J., Gao, and Yu, L. (2010). Fatty acid profile, thymoquinon content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. **LWT- Food Science and Technology**, 43, 1409-1413.

Maguire, L.S. O'sullivan, S.M., Gavline, K. O, connor, T.P. and O'Brain, N.M. (2004). Fatty acid profile, tocopherol, squalence and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. **International Journal of Food Science and Nutrition**, 55 (3), 171-178.

Makni, M., Fetoui, H., Gargouri, N.K., Garoui, E.M., Jaber, H., Makni, J., Boudawara, T. and Zeghal, N. (2008). Hypolipdemic and hepatoprotective effects of flax and pumpkin seed mixture rich in n-3 and n-6 fatty acids in hypercholesterolemic rats. **Food and Chemical Toxicology**, 46, 3714-3720.

Martin- Carron, N., Goni, I. Larrauri, J.A., Garcia – Alonso, A. and Saura- Calixto, F. (1999). Reduction In Serum Total and LDL cholesterol concentrations by a dietary fiber and Polyphenol – rich grape product in hypercholesterolemic rats. **Nutrition Research**, 19 (9), 1371-1381.

Mc cord, J.M. (2000). The evolution of free radicals and oxidative stress. **American Journal of Medical Science**, 108, 652-659.

Micallef, M.A and Garg, M.L. (2009) Beyond blood lipids: phytosterols, Statins and omega- 3 polyunsaturated fatty acid therapy for hyperlipidemia. **Journal of Nutritional Biochemistry**, 20, 927-939.

Moon, K.D. Back, S., Kim, J.H., Jean, S.M. and Choi, M.S. (2001). Safflower seed extract lowers plasma and hepatic lipids in rats fed high- cholesterol diet. **Nutrition Research,** 21, 895 – 904.

Moore, J., Hao, Z.,. Zhou, K., Luther, M. Costal, J. and Yu, L. (2005). Carotenoids, tocopherol, phenolic acid and antioxidant properties of Maryland- grown soft wheat. **Journal of Agricultural and Food Chemistry**, 53, 6649-6657.

Mudd, J.O., Borlaug, B.A., Johnston, P.V., Kral, B.G., Rouf., R., Blementhal, R.S. and Kwiterovich, P.O. (2007). Beyond Low-Density Lipoprotein Cholesterol. **Journal of the American College of Cardiology**, 50 (18), 1735-1741.

Nawar, W.W. (1996). Lipids. In: Fennema, O.R. (editor), **Food Chemistry**, (3rd ed.) 5, (pp. 225-319), New Yourk: Marcel Dekker, Inc.

Nevin, K.G and Rajamohan, T. (2008). Influence of Virgin coconut oil on blood coagulation factors, lipid levels and LDL oxidation in cholesterol fed Sprague- Dawley rats. **e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism**, 3, e1-e8.

Niki, E. (2010). Assessment of Antioxidant Capacity *in vitro* and *in vivo*. **Free Radical Biology and Medicine**, 49, 503-515.

Noguchi, N. and Niki, E. (1998). Dynamics of vitamin E action against LDL oxidation. **Free Radical Research**, 28, 561-572.

Nyam, k.L., Tan, C.P, Lai, O.M., Long, K. and Che Man, Y.B (2009). Physicochemical properties and bioactive compounds of selected seed oil. **LWT- Food Science and Technology**, 42, 1396-1403.

Nyiredy, S., Szucs, Z., Antus, S. and Samu, Z. (2008). New components from *Silybum Marianum* L. Fruits: A theory comes true. **Chromatographia Supplement**, 68, S5-S11.

Parker, T.D. Adams, D.A., Zhou, K., Harris, and Yu, L. (2003). Fatty acid composition and oxidative stability of cold-pressed edible seed oils. **Journal of Food Science**, 68, 1240-1243.

Parks, S.W. and Addis, P.B. (1986). Further investigation of oxidized cholesterol derivatives in heated fats. **Journal of Food Science**, 51 (5) 647 – 659.

Parry, J.W, Su, L., Luther, M., Zhou, K., Youraw ecz, M.P., Whittaker, P. and Yu, L. (2005). Fatty acid composition and Antioxidant properties of cold- pressed marionberry, Boysenberry, Red Raspberry and Blueberry Seed oils, **Journal of Agriculture and Food Chemistry**, 53, 566-573.

Parry, J.W., Cherg, Z., Moore, Jand Yu, L.L. (2008). Fatty acid composition, antioxidant properties and antiproliferative capacity of selected cold-pressed seed flours. **Journal of American Oil and Chemical Society**, 85, 457-464.

Parry, J.W., Hao, Z., Luther, M., Su, L., Zhou, K. and Yu, L.L. (2006). Characterization of cold-pressed onion, Parsley, Cardamom, Mullein, Roasted Pumpkin and Milk thistle seed oils. **Journal of American Oil and Chemical Society**, 83 (10) 847-854.

Pourmorad, F., Hosseinimehr, S.J. and Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants**. African Journal of Biotechnology**, 5 (11), 1142-1145.

Powers, S.K. and Lennon, S.L. (1999). Analysis of cellular responses to free radicals: Focus on exercise and skeletal Muscle. **Proceedings of the Nutrition Society**, 58, 1025 – 1033.

Ramasamy, K. and Agarwal, R. (2008). Multitargeted therapy of cancer by silymarin. **Cancer Lettures**, 269, 352-362.

Reeves, P.G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A Diet. **Journal of Nutrition**, 8385- 8415.

Roberfroid, M.B. (2000). Defining Functional Foods. In: Gibson G.R. and Williams, C, (editors)**, Functional Foods: Concept to Product.** (pp. 9-27), Cambridge: Wood head Publishing limited.

Rodrigues – Estrada, G. L. (2000). Chromatographic analysis of unsaponifiable compounds of olive oils and fat-containing foods. **Journal of Chromatography**, 881, 105-129.

Salter, A.M., Heather Mangiapane, E. H., Bennett, A.J., Bruce, J.S., Billett, M.A, Anderton, K.L., Marenah, C.B., Lawson, N. and White, D.A. (1998). The effect of different dietary fatty acids on lipoprotein metabolism: concentration- dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids. **British Journal of Nutrition**, 79, 195-202.

Satil, F., Azcan, N. and Baser, K.H.C. (2003). Fatty acid composition of pistachio nuts in Turkey. **Chemistry of Natural Compounds**, 39, 322-324.

Schuitz, R.M., and Liebman, M.N (2002). Proteins I: composition and structure. In: T.M. Devlin (Eds), **Textbook of Biochemistry with Clinical Correlations, (5th ed.),** (pp.23-85). New York: Wiley- liss.

Shaker, E., Mahmoud, H. and Mnaa, S. (2010). Silymarin, the antioxidant components and *Silybum Marianum* extracts prevent liver damage. **Food and Chemical Toxicology,** 48,803-806.

Shyu, Y.S. and Hwang, L.S. (2002). Antioxidative activity of the crude extract of lignan glycosides from unroasted Burma black sesame meal. **Food Research International**, 35, 357-365.

Simopoulos, A.P. (2002). The importance of the ratio of omega-6/ omega -3 essential fatty acids. **Biomed Pharmacother**, 56, 365-379.

Skottova, N., Vecera, R. Urban K, K., Vana, P., Walterova D. and Cvak, L. (2003). Effects of polyphenolic fraction of silymarin on lipoprotein profile in rats fed cholesterol- rich diets. **Pharmacological Research**, 47, 17-26.

Sobolova, L., Skottova, N., Vecera, R. and Urbanek, K. (2006). Effect of silymarin and its polyphemolic fraction on cholesterol absorption in rats. **Pharmacological Research**, 53, 104- 112.

Stadtman, E.R. and Levine, R.L (2003). Free radical mediated oxidation of free amino acids and amino acid residues in proteins. **Amino Acids**, 25, 207-218.

Subramaniam, S., Vaughn, K., Carrier, D.J. and Clausen, E.C. (2008). Pretreatment of milk thistle seed to increase the silymarian yield: An alternative to petroleum ether defatting. **Bioresource Technology**, 99, 2501-2506.

Tholstrup, T., Marckmann, P., Jespersen, J. and Sandstorm, B. (1994). Fat High in Stearic acid favorably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. **American Journal of Clinical Nutrition**, 59, 371-377.

Tuberose, C.I.G., Kowalczyk, A., Sarritzu, E. and Cebras, P. (2007). Determination of Antioxidant compounds and Antioxidant Activity in commercial Oil Seeds for Food use. **Food Chemistry**, 103, 1494-1501.

Tukan, S.K., Takruri, H. R and Ahmad, M.N. (2008). Use of Medicinal Plants and edible herbs in the Community of the Northern Badia of Jordan. **Jordan Journal for Agricultural Sciences**, 4 (4); 391-404.

Tukan, S.K., Takruri, H.R. and Al-Eisawi, D.M. (1998). The use of wild edible plants in the Jordanian diet. **International Journal of Food Sciences and Nutrition**, 49, 1889-1895.

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. **Free Radical Biology and Medicine**, 28, 1685-1696.

Urso, M.L. and Clarkson, P.M. (2003). Oxidative stress, exercise and antioxidant supplementation. **Toxicology**, 189, 41-54.

Visavadiya, N. and Narasimhacherya, A.V.R. (2008). Sesame as a hypocholestraemic and antioxidant dietary component. **Food and Chemical Toxicology**, 46, 1889-1895.

Vujasinovic, V., Djilas, S., Dimic, E. Romanic, R. and Takaci, A. (2010). Shelf-life of cold pressed pumpkin (*cucurbita pepo L.*) Seed oil obtained with a screw press. **Journal of American Oil and Chemical Society,** 87, 1497-1505.

Wagner, K.H., Kamal- Eldin, A. and Elmadfa, I. (2004). Gamma- Tocopherol- An underestimated vitamin?. **Annual Nutrition of Metabolism**, 48, 169-188.

Whent, M., Slavin, M., Kenworthy, W. and Yu, L. (2010). Potential relationships between fatty acid compositions and phytochemicals of selected low linolenic soybeans grown in Maryland. **Journal of American Oil and Chemical Society**, 87, 549-558.

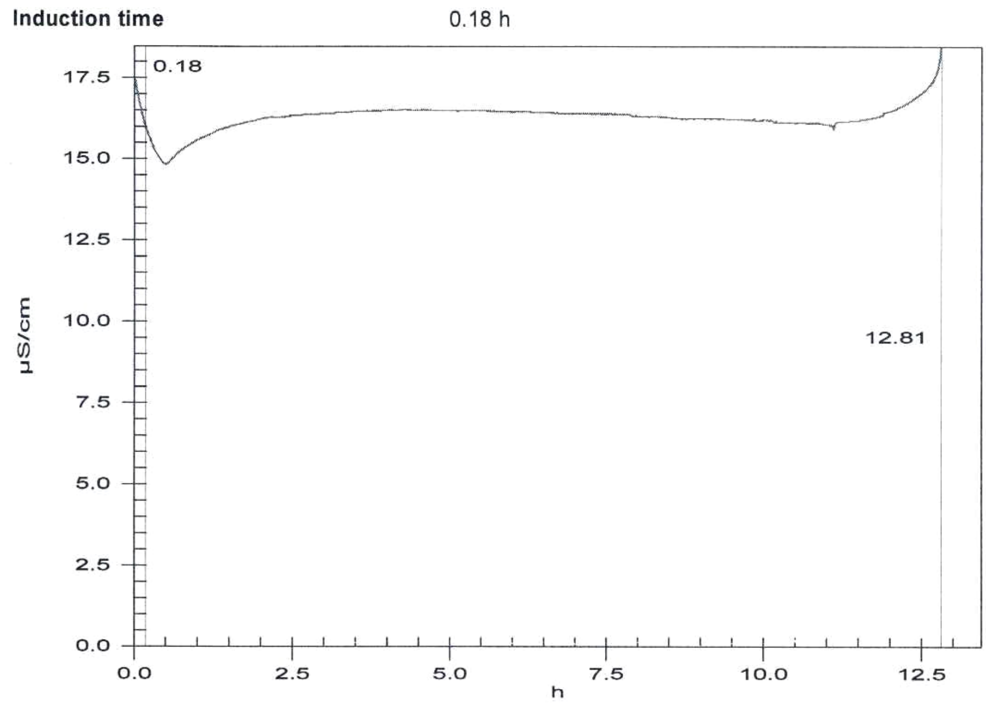
Whitney, E., Rolfes, S.R. (2008). **Understanding Nutrition,** (11ed.). Belmont, USA, Thomson Wadsworth.

Wilson, T.A., Ausman, L.M., Lawton, C.W., Hegsted, D.M. and Nicolosi, R.J. (2000). Comparative Cholesterol Lowering Properties of Vegetable Oils: Beyond Fatty Acids. **Journal of the American College of Nutrition**, 19 (5), 601-607.

Zohary, M. (1986). **Flora Palestinia**, pp. 221-224. Oxford University press, New Work.

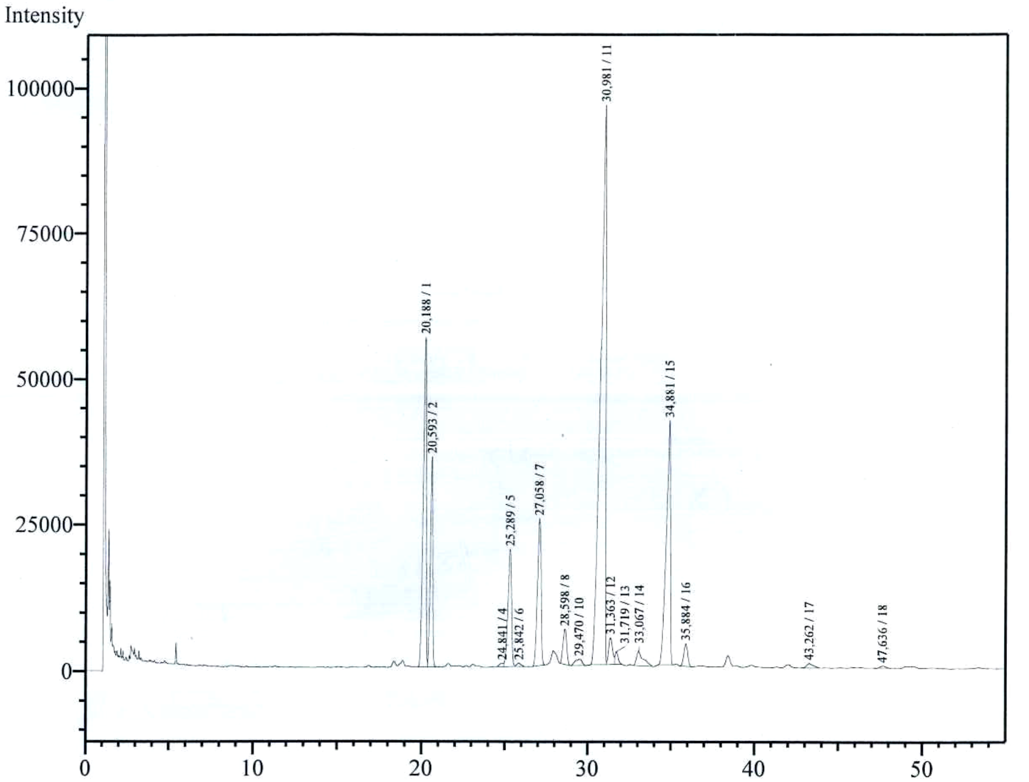
Zulueta, A., Esteve., M.J. and Frigola, A. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. **Food Chemistry**, 114, 310-316.

Appendix (1)



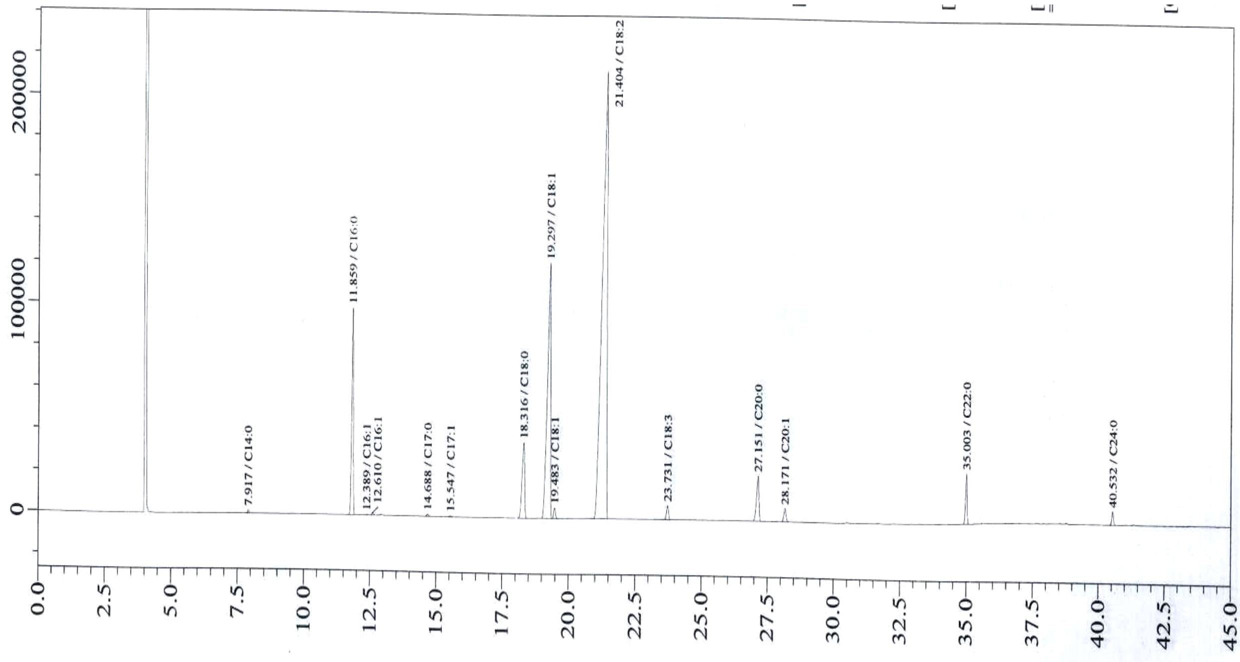
As an example, the OSI graph of one run of the cold-pressed MTSO (induction time of 12.88 hr at 100°C).

Appendix (2)



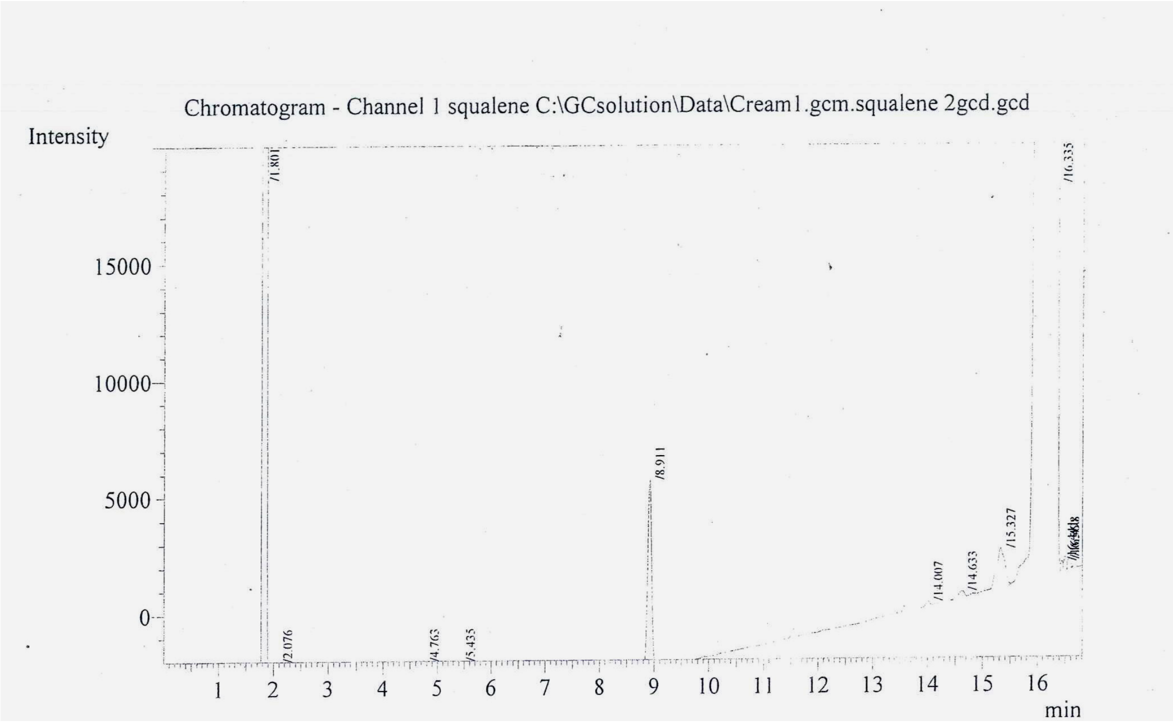
Gas chromatogram of fatty acids profile of cold-pressed MTSO

Appendix(3)



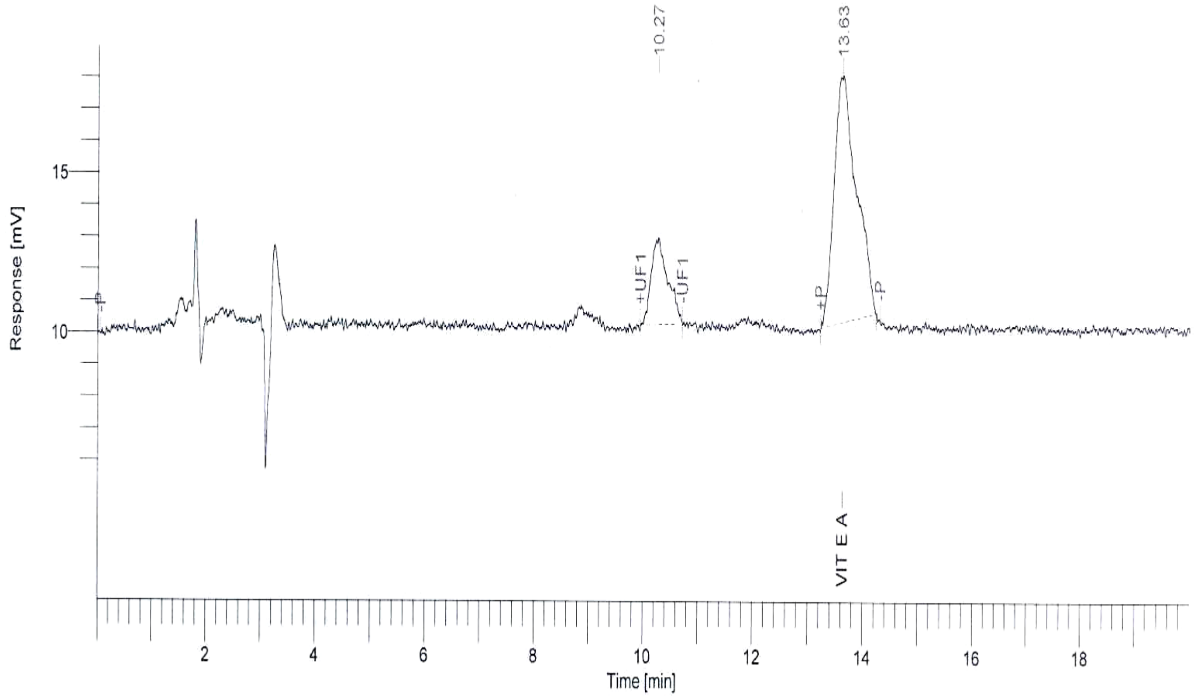
Gas chromatogram of phytosterols of cold-pressed MTSO

Appendix (4)



Gas chromatogram of squalene of cold-pressed MTSO

Appendix(5)



HPLC chromatogram of ∝-tocopherol of cold-pressed MTSO

الصفات الكيماوية لزيت بذور الخرفيش وتأثيره على صيغة ليبيدات مصل الدم والسعة المضادة للأكسدة في جرذان بالغة مغذاة بأطعمة مزودة بالكوليستيرول

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ملخص

هدفت هذه الدراسة إلى تحديد الخواص الكيماوية ومؤشرات الجودة لزيت بذور نبات الخرفيش المعصور بطريقة العصر البارد ودراسة التأثير طويل المدى لهذه الخواص الكيماوية في الجرذان وانعكاسه على محتوى مصل الدم من الشحميات والبروتينات الشحمية بالإضافة إلى السعة الكلية المضادة للأكسدة وناتج عملية التأكسد الداخلي للدهون .

تم قياس نسبة الحموضة ورقم البروكسيد بطريقة المعايرة الكيماوية القياسية ،وقياس مؤشر الاستقرار التأكسدي المحدد بجهاز الرانسمات وكذلك تم قياس محتوى الأحماض الدهنية والستيرولات النباتية والسكوالين باستخدام الكروماتوغرافيا الغازية (GLC)، بينما تم قياس الألفا- توكوفيرول باستخدام الكروماتوغرافيا السائلة عالية الكفاءة (HPLC) بالإضافة الى ذلك تم قياس المحتوى الكلي للفينولات وسعة كبح الجذور الحرة والسعة الكلية المضادة للأكسدة بجهاز المطياف الضوئي. ولدراسة مدى تأثير هذه المكونات الكيماوية في الجسم الحي تم دراسة التأثير طويل المدى لنسبتين من زيت بذور الخرفيش في الخلطات الغذائية الخاصة بالتجارب الحيوانية على محتوى مصل الدم من الشحميات والبروتينات الشحمية وناتج عملية التأكسد الداخلي للدهون (MDA) بالإضافة إلى السعة الكلية المضادة للتأكسد (TAC) في جرذان غُذيت أطعمة مزودة بالكوليسترول أو خالية منه. تم تقسيم أربعة وستين جرذاً ذكراً بالغاً من نوع سبراغ داولي الى ثمان مجموعات متساوية. غذيت كل مجموعة طعاماً يحتوي إما على نسبة 4 % أو 8 % من زيت بذور الخرفيش أو زيت الصويا (وجبة الشاهد) كلٍ على حده، مع أو بدون إضافة غرام واحد من الكوليسترول، لمدة اثنى عشر أسبوعاً. تم تحديد محتوى مصل الدم من الكوليسترول الكلي والبروتينات الشحمية عالية الكثافة ومنخفضة الكثافة والغليسيريدات الثلاثية والسعة الكلية المضاده للأكسدة باستعمال الطريقة اللونية الإنزيمية. وباستخدام جهاز المطياف الضوئي تم قياس ناتج عملية التأكسد الداخلي للدهون (MDA). كما تم قياس أوزان الجسم والكبد إضافة إلى كميات استهلاك الغذاء.

تبين من الخواص الكيماوية ومؤشرات الجودة للزيت ما يلي: بلغت نسبة الحموضة ورقم البروكسيد 0.64% و 0.34 مل مكافئ جزيئ الاوكسجين/ كغم زيت على التوالي ومؤشر الاستقرار التأكسدي 55.7 ساعة و 12.9 ساعة عند درجة حرارة 80 مْ و 100 مْ على التوالي. وكانت نسبة الأحماض الدهنية 19.5 % و 22.9 % و 57.6 % لكلٍ من الأحماض الدهنية المشبعة وحيدة اللاإشباع و عديدة اللاإشباع على التوالي ومحتوى الستيرولات النباتية 2520 ملغم/ كغم والسكوالين 9.35 ملغم/كغم. وكان محتوى الألفا- توكوفيرول 237.4 ملغم/كغم. وبلغ المحتوى الكلي للفينولات 1.16 ملغم/غم زيت مقدراً بمكافئ حمض الجاليك. بالإضافة إلى ذلك وجد أن سعة كبح الجذور الحرة بإختبار المستخلص الميثيلي للزيت على الجذر الحر DPPH بنسبة 50% من قدرة الكبح لتركيز المستخلص الميثيلي بلغت (3.34 ملغم/مل) بينما بلغت قيمة السعة الكلية المضادة للأكسدة (2.29 مل مول/لتر). وجد أن الأطعمة المزودة بالكوليسترول والخالية منه والتي تحتوى على 4% و8% زيت، لم تؤد إلى إحداث تغيُر ذي دلالة معنوية (P>0.05) على أوزان الجسم والكبد وكميات استهلاك الغذاء التراكمية وكفاءة الغذاء، باستثناء أوزان الكبد التي كانت أعلى بصورة معنوية (P<0.05) في المجموعة المغذاة بوجبة تحتوي على 8% زيت بذور الخرفيش و 8% زيت صويا (مجموعة الشاهد) والمزودة بالكوليسترول بالمقارنة مع المجموعات الأخرى. وعند تحييد دور كلٍ من نوع الزيت وفيما بعد نسبة الزيت المضاف بقيت النتيجة السابقة نفسها. وعند تحييد دور الكوليسترول لم يكن ثمة فروقات ذات دلالة

معنوية (P>0.05) في تلك المتغيرات، مما يبرز تأثير الكوليسترول على زيادة أوزان الكبد. فيما يتعلق بالبروتينات الشحمية كان هناك فروق معنوية في الكوليسترول الكلي (P<0.05) في وجبة 8% زيت الخرفيش و 8% وجبة الشاهد المزودة بالكوليسترول وذلك عند مقارنتها بوجبة الشاهد 8% الخالية من الكوليسترول. وكذلك اختلفت البروتينات الشحمية منخفضة الكثافة معنوياً للمجموعات المزودة بالكوليسترول (P<0.05) عن المجموعات الخالية من الكوليسترول. أما بالنسبة للبروتينات الشحمية عالية الكثافة فكانت أعلى لدى الجرذان المزودة بغذاء خالٍ من الكوليسترول والمحتوي على 4% و 8% من زيت الخرفيش وذلك عند مقارنتها بالوجبات المزودة بالكوليسترول. وبالتالى كان هناك فرق معنوي واضح (P<0.05) عند حساب نسبة البروتينات الشحمية عالية الكثافة الى منخفضة الكثافة للوجبات الخالية من الكوليستيرول بالمقارنة مع الوجبات المزودة بالكوليستيرول، وهذا انعكس على مؤشر إحداث تصلب الشرايين حيث كان عالياً بدلالة معنوية (P<0.05) عند 8 % زيت في الوجبة المزودة بالكوليسترول مقارنة بالوجبات الخالية من الكوليسترول. وعند تحييد نوع الزيت وأخذ نسبته المضافه مع وجود الكوليسترول في الوجبات بعين الاعتبار، تكون المؤشرات التالية منخفضة معنوياً (P<0.05) عند مستوى 4% زيت: الكوليستيرول الكلي والبروتينات الشحمية منخفضة الكثافة ونسبة الكوليسترول الكلي الى الغليسيريدات الثلاثية و مؤشر إحداث تصلب الشرايين وذلك بالمقارنة مع تركيز زيت 8 % لتلك القيم في الدم. وفيما يتعلق بالغليسيريدات الثلاثية في مصل الدم لم يكن هناك اختلاف معنوي (P>0.05) في جميع الوجبات باستثناء وجبة الشاهد الخالية من الكوليستيرول عند 4 % والتي أعطت بدورها أعلى قيمة للغليسيريدات الثلاثية بمقارنتها بجميع الوجبات، وبقيَ هذا التأثير واضحاً عند 4% حتى عند تحييد تأثير نوع الزيت.

بالنسبة لناتج عملية التأكسد للدهون(MDA) كانت القيمة أعلى عند 8% لوجبة الشاهد الخالية من الكوليسترول مقارنة مع الوجبات الأخرى الخالية من الكوليسترول. بالإضافة إلى ذلك كانت القيمة أيضا عالية عند 8% لمجموعة الشاهد التي تحتوي على الكوليسترول مقارنة مع الوجبة 8% المحتوية على زيت الخرفيش والمحتوية على الكوليسترول (P<0.05) وحدث ذلك أيضاً عند تحييد وجود الكوليسترول. بينما بوجود الكوليسترول في الوجبات المحتوية على 4% و8%، كان مستوى ناتج التأكسد للدهون مرتفعاً (P<0.05) مقارنة مع 4% بدون كوليسترول. وعند أخذ تأثير نوع الزيت بوجود الكوليستيرول (مع تحييد دور نسبة الزيت) كان المستوى أيضا مرتفعاً في وجبة الشاهد مع وبدون الكوليسترول، وهذا يشير بأن أعلى مستوى لتأكسد الدهون كان لوجبة الشاهد المحتوية على 8% زيت صويا مع وبدون الكوليسترول. وفيما يتعلق بقيم السعة الكلية المضادة للأكسدة، كانت القيمة أعلى (P<0.05) لوجبة زيت الخرفيش 4% الخالية من الكوليستيرول وذلك بمقارنتها بوجبة الشاهد 8 % والخالية من الكولستيرول وكذلك بالمقارنة مع وجبة زيت الخرفيش 4% المحتوية على الكوليسترول. وبتحييد نسبة الزيت المضاف، وجد أن السعة الكلية المضادة للأكسدة مرتفعه لوجبة زيت الخرفيش الخالية من الكوليسترول .

وتقترح هذه النتائج أن زيت بذور الخرفيش المعصور عصرا بارداً يُعد مصدراً للأحماض الدهنية وحيدة اللاإشباع والمتعددة اللاإشباع والستيرولات النباتية والمركبات الفينولية بالاضافة الى مضادات التأكسد الطبيعية مما يساعد في استخدامه في الخلطات والمستحضرات الغذائية الصحية وإمكانية خلطه بزيوت نباتيه أخرى لرفع قيمتها وتحسينها أو استخدامه بمفرده. واعتماداً على هذه النتائج أيضاً، كانت نسبة الزيت ووجود الكوليستيرول في الغذاء هما العاملان المؤثران على صيغة ليبيدات الدم ومؤشر إحداث تصلب الشرايين وتأكسد الدهون والسعة المضادة للتأكسد. وبالتالي يمكن القول أن زيت بذور الخرفيش وزيت الصويا(الشاهد) كان لهما التأثير نفسه في تلك المتغيرات، مع ملاحظة أن زيت بذور الخرفيش كان أفضل بصورة واضحة من زيت الصويا فيما يتعلق بعملية تأكسد الدهون داخل الجسم الحي .